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Chapter 3: Effect of Ethanol and MtBE on BTEX Biodegradation in the Saturated Zone: Kinetic Studies

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Summary (Effect of Ethanol and MtBE on BTEX Biodegradation in the Saturated Zone: Kinetic Studies)

There is substantial knowledge about many of the mechanisms affecting saturated zone transport of gasoline containing ethanol. Microbially-mediated processes appear to dominate the fate and transport of gasoline components in the presence of ethanol, but additional research was needed to better understand the impact that ethanol may have on benzene, toluene, ethylbenzene, and xylene (BTEX) biodegradation kinetics. The research presented in this chapter found that:

1. BTEX and ethanol were typically degraded more rapidly in microcosms that used previously contaminated aquifer material, although previous exposure did not always result in high degradation activity.
2. Methyl tertiary butyl ether (MtBE) was not degraded within 100 days under any conditions, and did not affect BTEX or ethanol degradation patterns.
3. Ethanol was typically degraded before BTEX compounds, and had a variable effect on BTEX degradation as a function of electron-accepting conditions and bacterial source. This is best illustrated with toluene, which was the most commonly degraded of the BTEX compounds. In some cases, ethanol retarded toluene degradation, but it occasionally enhanced toluene degradation in microcosms with electron acceptors supplied in excess. This enhancement may be attributable to the fortuitous growth of toluene-degrading bacteria during ethanol degradation.

As part of this study, aquifer columns were also used to characterize ethanol, MtBE, and BTEX migration and biodegradation in a flow-through system simulating natural attenuation. The results from these column studies indicate that:

- Both ethanol and MtBE could enhance dissolved BTEX mobility by exerting a cosolvent effect that decreases sorption-related retardation. This effect, however, is concentration-dependent and was not observed when ethanol or MtBE was added (at 1%) continuously with BTEX to sterile aquifer columns. However, a significant decrease in BTEX retardation was observed with 50% ethanol, suggesting that neat ethanol spills in bulk terminals could facilitate the migration of pre-existing contamination.
- The preferential degradation of ethanol and the accompanying depletion of oxygen and other electron acceptors suggest that ethanol could hinder the natural attenuation of BTEX plumes. Using non-sterile columns packed with soil material, ethanol (106 mg/L) was degraded rapidly and exerted a high demand for nutrients and electron acceptors that could otherwise have been used for BTEX degradation. MtBE (9 mg/L), on the other hand, was not degraded and did not affect BTEX degradation.

The results presented in this chapter are particularly important for the fate of benzene, which is the most toxic and the most recalcitrant of the BTEX compounds under anaerobic conditions. Nevertheless, it is unknown to what extent ethanol would increase the distance that benzene

migrates before being attenuated to acceptable concentrations by natural processes. Therefore, the following studies should be conducted to quantify the effect of ethanol on plume length and improve our risk assessment capabilities:

- A controlled release and additional study of field sites: One major concern is that ethanol could increase the distance that BTEX compounds migrate before being attenuated to acceptable concentrations by natural processes. Nevertheless, there is considerable uncertainty regarding the magnitude and significance of this potential impact. Therefore, field-scale and modeling studies should be conducted to quantify the effect of ethanol on plume length. Such studies could include controlled-release (field) experiments and statistical analyses of LUFT site data with and without ethanol. Controlled-release studies should be multidisciplinary, and could benefit from incorporation of the microbial ecology technique developed for this project (Chapter 4, Beller *et al.*, 2001).
- Better integration of ethanol degradation kinetics into models: Mathematical fate and transport models should also be developed and calibrated to integrate the negative effects of ethanol on BTEX degradation (e.g., electron acceptor depletion and/or repression of BTEX metabolic flux) with potential positive effects (e.g., enhanced bacterial growth). Such models would be useful for risk assessment and management purposes.
- Improved anaerobic biostimulation strategies: Longer BTEX plumes represent a greater risk of exposure to potential downgradient receptors, which could result in decreased acceptability of natural attenuation as a remedial approach at some sites. This could stimulate a shift of cleanup decisions towards engineered remediation approaches. Although the most common engineered bioremediation approaches used for BTEX cleanup are aerobic, introducing sufficient oxygen to meet the high oxygen demand exerted by ethanol will likely be technically difficult and prohibitively expensive. Therefore, anaerobic biostimulation strategies should be considered. However, the lack of field experience with enhanced anaerobic bioremediation approaches for BTEX contamination will require the refinement and demonstration of suitable approaches. These could include the addition of nitrate to increase the electron acceptor pool (in a manner that does not create toxicity or clogging problems), and bioaugmentation with anaerobic cultures that can degrade benzene, which is relatively recalcitrant under anaerobic conditions.
- Improved characterization of methane, and volatile fatty acids at ethanol release sites: Neat ethanol spills and some gasohol releases could pose an explosion risk when site-specific conditions favor extensive methanogenesis and methane accumulation. In addition, ethanol-derived acetate and other volatile fatty acids could cause a decrease in pH (thus hindering biodegradation processes) and create taste and odor problems. Therefore, site characterization protocols should include methane and volatile fatty acid analyses near the source zone. Aesthetic impacts to groundwater quality could also be created by reductive dissolution of iron and manganese caused by metal-reducing bacteria feeding on ethanol. Therefore, dissolved metal analyses should also be considered.

3. Effect of Ethanol and MtBE on BTEX Biodegradation in the Saturated Zone: Kinetic Studies

3.1. Introduction

Monoaromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylenes (BTEX) are ubiquitous groundwater pollutants commonly associated with petroleum product releases. As of the end of the year 2000, 412,392 confirmed releases had been reported in the U.S. from leaking underground tanks alone (Mattick, 2000). Understanding the factors that affect the fate and transport of BTEX compounds in aquifers is of paramount importance for risk assessment and corrective action purposes. While significant advances have been made towards understanding the genetic and biochemical basis of BTEX biodegradation, little attention has been given to how differences in gasoline formulation affect such natural attenuation processes. In this regard, there is a recent initiative being implemented to phase out methyl tertiary butyl ether (MtBE) as a gasoline oxygenate (Federal Register, 2000), due to its recalcitrance, ability to rapidly impact drinking water sources, and low taste and odor thresholds (<5-40 ppb). The most likely candidate to replace MtBE (which accounts for 80% of current oxygenate use) is ethanol (currently accounting for 15% of oxygenate use [Powers *et al.*, 2001]). Ethanol is a renewable resource that can serve as a substitute fuel for imported oil. Therefore, an increase in the use of ethanol as a gasoline additive seems imminent, and a better understanding of its effects on BTEX migration and natural attenuation is warranted. There is also considerable interest in the State of California about how ethanol might affect the natural attenuation of pre-existing MtBE contamination, which motivated the incorporation of MtBE in this study of substrate interactions between BTEX and ethanol.

To understand how ethanol might affect the natural attenuation of BTEX and MtBE, we need to address some knowledge gaps related to microbial ecology and fate and transport phenomena. Natural attenuation relies heavily on anaerobic biodegradation processes (Rifai *et al.*, 1995). In such cases, indigenous microorganisms often degrade BTEX using electron acceptors preferentially in order of decreasing oxidation potential (Chapelle, 1993). Sequential depletion of electron acceptors could lead to successive transitions from aerobic to denitrifying, iron-reducing, sulfate-reducing, and methanogenic conditions. Ethanol would likely contribute to the depletion of electron acceptor pools during its microbial degradation (Corseuil *et al.*, 1998). However, little is known about the extent to which ethanol affects the related microbial population shifts and geochemical transitions. Such transitions are important to study because they affect both BTEX degradation and migration rates. For example, both the changes in electron acceptor availability and the presence of easily degradable ethanol could affect catabolic diversity and the relative abundance of specific BTEX-degrading bacteria. In addition, ethanol may stimulate some microbial processes that affect aquifer porosity and hydraulic conductivity (e.g., mineral precipitation or dissolution, and N_2 or CH_4 gas generation). Also, exposure history to BTEX, MtBE, and ethanol in soil may be important to consider, with the presumption that microbial communities with previous exposure to contaminants are better adapted to degradation.

In this study, we conducted aquifer microcosm and flow-through column studies to address the following questions:

a) How do ethanol and MtBE affect BTEX biodegradation patterns under different electron-accepting conditions, and how do such effects differ from one site to another?

b) How does ethanol affect adaptation mechanisms related to microbial population shifts, and to what extent do such changes in microbial ecology explain the observed biodegradation patterns? (see Chapter 4; Beller *et al.*, 2001)

c) Does ethanol enhance BTEX migration by decreasing sorption-related retardation?, and

d) What might be the overall effect of ethanol on BTEX and MtBE natural attenuation?

3.2. Scope of Work and Specific Objectives

This project compared the effects of ethanol versus MtBE on the degradation of BTEX compounds under different electron-accepting conditions commonly encountered at sites undergoing natural attenuation (i.e., aerobic, denitrifying, iron-reducing, sulfate-reducing, and methanogenic conditions). Emphasis was placed on studying substrate interactions between ethanol, MtBE, and BTEX. Four different aquifer materials of varying exposure history were used to investigate response variability. Material from the Travis Air Force Base (AFB), CA, and Sacramento, CA, sites had BTEX and MtBE exposure histories, whereas, the Northwest Terminal site, OR, had been exposed to BTEX and ethanol. Material from the Tracy site, CA, functioned as a control since it had no exposure to BTEX, MtBE, or ethanol (Tables 3-1 and 3-2).

Specific objectives included:

1. To conduct microcosm studies to provide as much predictive information as possible about the effects of ethanol on BTEX and MtBE degradation, by surveying sites with different exposure histories and under a range of electron-accepting conditions. Emphasis was placed on monitoring changes in the concentration of various analytes (e.g., ethanol, BTEX, acetate, methane, pH, dissolved oxygen, nitrate, nitrite, ferrous iron, and sulfate) and determining degradation rates and lag times before degradation commenced.

2. In parallel with this microcosm study, to construct additional (sacrificial) microcosms for molecular analysis of microbial population shifts due to exposure to BTEX and/or ethanol. This exercise also served as an evaluation of a novel molecular biology method to assess anaerobic BTEX bioremediation (see Chapter 4; Beller *et al.*, 2001).

3. To perform aquifer column studies to characterize ethanol, MtBE, and BTEX migration and biodegradation and geochemical transitions in a flow-through system simulating natural attenuation. This study was conducted to provide support for the microcosm results, and was run with aquifer material from only one site (Travis AFB) because of logistical and cost constraints. Additional (sterile) columns were packed with material from the Northwest Terminal site to characterize the effect of ethanol on sorption-related retardation of dissolved BTEX compounds.

3.3. Methodology

3.3.1. Microcosm Degradation Assays

3.3.1.1. General Approach

Aquifer microcosms were used to investigate how exposure history and electron-accepting conditions affect the nature and extent of substrate interactions between BTEX, ethanol, and MtBE. Microcosms were prepared using aquifer materials from four different sites that differed in exposure history to BTEX, MtBE, and ethanol (Tables 3-1 and 3-2). For each site, microcosms were prepared under aerobic and anaerobic conditions.

Aerobic microcosms were prepared by adding 20 g of soil and 80 mL of aerated mineral medium (described in Section 3.3.1.2) to 250-mL amber bottles. Microcosms were prepared in triplicate and capped with Mini-nert valves. Air in the headspace was replaced with oxygen. Four sets of aerobic microcosms were prepared: BTEX alone, BTEX plus ethanol, BTEX plus MtBE, and BTEX plus ethanol and MtBE. Abiotic controls were prepared with all compounds added, and were poisoned with a commercial biocide (Kathon CG/ICP, Supelco, diluted 1:100). All microcosms were incubated under quiescent conditions at 28^{o}C, and aqueous samples were collected periodically to determine changes in contaminant concentrations.

Anaerobic microcosms were prepared similarly, and were incubated under quiescent conditions at 25 ± 3 ^{o}C inside an anaerobic chamber with an atmosphere composed of N_2 (80%), CO_2 (10%) and H_2 (10%). The mineral medium for anaerobic microcosms was deoxygenated and amended with appropriate electron acceptors as described in Section 3.3.1.2. Briefly, nitrate, ferric iron, or sulfate was added to denitrifying, iron-reducing, and sulfate-reducing microcosms, respectively. Additional anaerobic microcosms were prepared without supplemental electron acceptors to facilitate the establishment of methanogenic conditions.

It should be pointed out that there is some uncertainty about the electron-accepting conditions responsible for degradation in "methanogenic" microcosms. The quotation marks reflect this uncertainty, which is due to two confounding factors. First, all microcosms contained about 22 mg/L of background sulfate (present in the mineral medium). This raises the possibility that sulfate-reducing bacteria were at least partially responsible for degradation activity observed in "methanogenic" microcosms. Secondly, although methane was detected in the headspace of "methanogenic" microcosms, these data were confounded by fluctuating background levels in the anaerobic chamber, because methane was occasionally released from aquifer cores that were stored and opened during the course of the experiment. Therefore, we have not included the methane data because they may be unrepresentative. These data, however, can be found in the appendices.

As was the case for the aerobic microcosms, different treatment sets were prepared in triplicate for each electron-accepting condition to study BTEX degradation in the presence and absence of ethanol and MtBE. Anaerobic controls were also prepared by adding all tested compounds and electron acceptors, and were poisoned with the commercial biocide Kathon CG/ICP, Supelco (10 mL/L). Overall, 184 microcosms (aerobic and anaerobic) were prepared for this experiment.

The removal of a compound from viable microcosms but not from sterile controls was taken as evidence of biodegradation. Some anaerobic sets (including controls) exhibited BTEX losses, possibly due to volatilization or other abiotic processes. In such cases, the ratio of toluene to benzene was considered as an additional criterion to determine if degradation had occurred. The presumption was that this ratio would decrease significantly as a result of biodegradation (e.g., by 50% or more) because benzene is relatively recalcitrant, whereas toluene is commonly reported to degrade under anaerobic conditions (Corseuil *et al.*, 1998; Heider *et al.*, 1998).

For cases where biodegradation was unequivocally established, biodegradation patterns were characterized by determining lag periods and first-order degradation rate coefficients. The lag period was determined as the time during which contaminant concentrations remained constant or did not decrease significantly relative to controls. The first-order rate coefficient (k) was determined by fitting a simple exponential decay model ($C = C_o e^{-kt}$) to concentration (C) versus time (t) data obtained after the lag period (Figure 3-1). This value was then corrected for volatile losses by subtracting the k-value obtained for the controls.

3.3.1.2. Mineral Media for Microcosms

The basal medium used to prepare anaerobic microcosms was bicarbonate-buffered synthetic groundwater prepared as described by von Gunten and Zobrist (1993), except that 7.4 mM bicarbonate was added. This concentration is higher than the median bicarbonate concentration found in terrestrial waters (ca. 4 mM) (Stumm and Morgan, 1996), and was used to provide additional buffering capacity. In addition, NH_4Cl substituted for $NaNO_3$ at 0.3 mM to provide a nitrogen source that could not be used as electron acceptor, and the phosphate concentration was increased from 0.6 to 20 μM to ensure that P was not limiting. The medium contained (in mg per liter of deionized water): K_2SO_4 (40); NH_4Cl (16); $CaCl_2$ (6.7); $MgCl_2.6H_2O$ (12); K_2HPO_4 (3.5); H_3BO_3 (0.0004); $Ni(NO_3)_2.6H_2O$ (0.002); $CuSO_4.5H_2O$ (0.002); $ZnSO_4.7H_2O$ (0.002); $CoSO_4.7H_2O$ (0.002); and $(NH_4)_6Mo_7O_{24}$ (0.001).

The medium was autoclaved for 15 minutes at 120°C and purged for two hours with N_2/CO_2 (80/20) to remove dissolved oxygen prior to being transferred to the anaerobic chamber, where it was equilibrated with a $N_2/CO_2/H_2$ (80/10/10; v:v:v) atmosphere for three days before starting the experiment. The pH of the medium was 7.4 at that time. The medium was amended with different electron acceptors prior to BTEX, ethanol, and/or MtBE addition. For denitrifying microcosms, the medium contained ca. 5.3 mM nitrate. The medium for iron (III)-amended microcosms had 250 mM amorphous ferric oxyhydroxide, prepared as described by Lovley and Phillips (1986). The medium for sulfate-reducing microcosms contained about 4 mM sulfate. "Methanogenic" microcosms were not amended with additional electron acceptors other than the background sulfate (0.23 mM) and bicarbonate (7.4 mM) present in the medium.

The same basal mineral medium recipe was used for aerobic microcosms, except that $NaHCO_3$ was replaced by KH_2PO_4 buffer (3.9 mM, pH 7.0) since there was no need to provide bicarbonate as an electron acceptor. The aerobic medium was also purged with pure O_2 for 30 minutes prior to transferring into the microcosms.

3.3.1.3. Travis AFB Microcosms

Aquifer material was obtained from a plume containing BTEX and MtBE (Table 3-2) in Travis AFB, CA. Aquifer sediment samples were collected from a location near monitoring well

MW-135 at Travis AFB on March 24, 2000, using Geoprobe Direct Push sampling. Three borings were taken, and subsamples for microcosm and column construction were taken from depths of 13.5 to 22 ft (depth to groundwater was approximately 9.4 ft). The aquifer material in acrylic liners was shipped on ice and stored at 4°C. Similar to other microcosm sets, the aquifer material (Table 3-1) was drained for 2 days inside an anaerobic chamber (80 % N_2/10 % CO_2/10 % H_2) and homogenized prior to transferring it into the microcosms. Initial concentrations of BTEX, ethanol, and MtBE are given in Table 3-3, and electron acceptor amendments are summarized in Table 3-4.

3.3.1.4. Tracy Site Microcosms

Aquifer material was obtained from the Tracy Fish Facility (near Tracy, CA), which had no known previous exposure to BTEX, ethanol, or MtBE (Table 3-2). Aquifer sediment samples were collected in May, 2000 using a Standard Penetration Test (SPT) tool. Initial concentrations of BTEX and ethanol are given in Table 3-5, and electron acceptor amendments are summarized in Table 3-6. Because of logistic and cost constraints, MtBE was not used in microcosms from this control site.

3.3.1.5. Sacramento Site Microcosms

Aquifer core samples were obtained from a plume containing BTEX and MtBE in Sacramento, CA (Table 3-2) on May 3, 2000 by using a split-spoon sampler. The material (described in Table 3-1) was collected from below the water table in 2.5-inch diameter brass sleeves and refrigerated at 4°C. Initial BTEX, ethanol, and MtBE concentrations are summarized in Table 3-7, and electron acceptor amendments in Table 3-8. Note that no Fe(III)-amended microcosms were prepared for this site.

3.3.1.6. Northwest Terminal Site Microcosms

Core samples were collected from the Northwest Terminal in Oregon, where a 19,000-gallon release of neat ethanol occurred over pre-existing BTEX contamination (Table 3-2). The aquifer material (described in Table 3-2) was collected on July 11, 2000 by using a hand-held percussive hammer to advance a 1″-diameter GeoProbe rod. Continuous samples were collected below the water table at depths between 6 and 12 feet below ground surface. Aquifer sediment in capped, acrylic liners was shipped on ice and stored at 4°C upon receipt. Because some treatments required the absence of ethanol, this aquifer material was removed from the core and allowed to vent inside the anaerobic chamber for three days to remove residual ethanol by volatilization. This material had no known previous exposure to MtBE, so MtBE was excluded from Northwest Terminal site microcosms. Initial BTEX and ethanol concentrations and electron acceptor amendments are summarized in Tables 3-9 and 3-10, respectively.

3.4. Sacrificial Microcosms for Molecular Analysis of the Microbial Community

Replicate microcosm sets were prepared for sacrifice in order to characterize microbial population shifts during biodegradation of BTEX/ethanol mixtures. This analysis was conducted at Lawrence Livermore National Laboratory (LLNL), and is described in Chapter 4 (Beller *et al*., 2001). "Sacrificial" microcosms were smaller, but were prepared similarly and at the same time

as the previously described microcosms (Table 3-11). These microcosms were prepared with 10 g of drained aquifer material and 40-mL medium in 125-mL amber bottles capped with Mini-nert valves. For each site and electron accepting condition tested, two microcosm sets were prepared (with and without ethanol) with four replicates per set. One additional quadruplicate set was prepared without BTEX or ethanol to characterize the initial microbial community. Overall, 176 sacrificial microcosms (aerobic and anaerobic) were prepared for this experiment.

For each aquifer material and electron-accepting condition tested, four microcosms were sacrificed following the removal of ethanol (i.e., two from the set with ethanol and two from the set without ethanol). This facilitated studying how growth on ethanol affects the relative abundance of BTEX-degrading bacteria (see Chapter 4; Beller *et al.*, 2001). The remaining four microcosms were sacrificed after the first BTEX compound (usually toluene) was degraded. Since BTEX degradation was not necessarily concurrent in sets with and without ethanol, two microcosms from each set were sacrificed following BTEX degradation in that set. All microcosms were sampled prior to termination to confirm that ethanol or BTEX had been degraded. This analysis was conducted only after degradation had been observed in the larger microcosms described in the previous section.

Prior to shipping for molecular analysis, the contents of the microcosms were transferred to 35-mL tubes for centrifugation in a Marathon 21K/Br (Fisher Scientific) apparatus at 4^{o}C and 10,000 rpm for 15 min. The liquid was then decanted and the remaining aquifer material was frozen and stored at -40 ^{o}C until shipment to the LLNL in coolers filled with ice.

3.5. Aquifer Columns

3.5.1. Breakthrough Experiments

Small aquifer columns were used to conduct breakthrough studies and compare the effects of ethanol and MtBE on sorption-related BTEX retardation factors. Three glass columns (Kontes, 15 cm long, 1 cm I.D.) were packed with uncontaminated soil (f_{oc} = 0.024) collected near the Northwest Terminal site. The columns were packed as described elsewhere (McCarty, 1987; Alvarez *et al.*, 1998), to ensure that no air bubbles were trapped. All tubes and fittings in the flow train were made of Teflon or were Teflon-lined to minimize sorption and volatilization losses. The feeds were dispensed from 100-mL gas-tight syringes (SGE, Austin, TX) at a constant flow of 1 mL/h using a syringe pump (Harvard, South Natick, MA). The tubing was adapted for sampling with a 1/4″ 28-adapter male Luer lock fitting and a thin (30-gauge) disposable syringe needle. The columns were fed continuously with benzene (5 mg/L), which is the most toxic of the BTEX compounds, toluene (5 mg/L), which is the compound most commonly reported to biodegrade, and *o*-xylene (3 mg/L), which is frequently the most recalcitrant compound under aerobic conditions (Gulensoy and Alvarez, 1999). These compounds were dissolved in the mineral medium described in Table 3-12 (pH ca.7). Two columns were also amended with either ethanol or MtBE (at 10,000 mg/L each) and the other column (without oxygenate amendment) was used as a baseline to evaluate changes in retardation factors. All columns were poisoned with a biocide (Kanthon CG/IPC [Supelco©, Bellefonte, PA], diluted 1:100) to eliminate confounding effects due to biodegradation and isolate the effects of ethanol on sorption-related retardation. Samples were taken every hour by attaching the effluent end of the column train to collection vials (5 mL CG vial, Kimble) closed

previously with Teflon-lined rubber septa and aluminum crimps. The samples were analyzed immediately after collection for BTX, MtBE, and/or ethanol using gas chromatography.

The hydraulic characteristics of the columns were determined from bromide tracer studies. A carbonate solution (1,000 mg/L) spiked with potassium bromide (1,500 mg/L) was fed continuously at 1 mL/h. The bromide breakthrough curve was used to calculate the effective porosity (η_e), dispersion coefficient (D) and retardation factors (R_f) by fitting the breakthrough data to the solution of the 1-D advection-dispersion equation (Domenico and Schwartz, 1998) Equation (3-1):

$$C = \left(\frac{Co}{2}\right) \operatorname{erfc}\left[\frac{\left(R_f x - \left(\frac{Q}{A\eta_e}\right)t\right)}{2\sqrt{DR_f t}}\right] + \exp\left[\frac{\left(\frac{Q}{A\eta_e}\right)x}{D}\right] \operatorname{erfc}\left[\frac{\left(R_f x + \left(\frac{Q}{A\eta_e}\right)t\right)}{2\sqrt{DR_f t}}\right] \quad \textbf{(3-1)}$$

Where C is the effluent concentration, C_o is the influent concentration, x is the column length, t is the elapsed time, Q is the flow rate, A is the cross-sectional area, and erfc is the complementary error function. The effective porosity was estimated to be 0.45, yielding a pore velocity of 2.8 cm/h. One pore volume was exchanged every 5.7 hours. The dispersion coefficient was estimated to be 0.7 cm^2/h. Retardation factors were 1.0 for ethanol and MtBE, 1.6 for benzene, 2.8 for toluene, and 11.3 for *o*-xylene.

To determine how ethanol or MtBE might affect the natural attenuation of the leading edge of a BTEX plume, this column experiment was repeated under non-sterile conditions using aquifer material from the Travis AFB site (Table 3-2). Four columns were packed and operated similarly, except that the flow rate was decreased to 3 mL/h resulting in a hydraulic retention time of 2.66 hours. For all columns, influent concentrations were about 5.0 mg/L for benzene, 3.0 mg/L for toluene, 3.0 mg/L for ethylbenzene, 2.0 mg/L for *m*+*p*-xylenes, and 3 mg/L for *o*-xylene. One column was amended with BTEX alone, the second column was fed BTEX plus 150 mg/L ethanol, the third column received BTEX plus 12 mg/L MtBE, and the fourth column was used as a sterile control and was amended with all compounds (at the previously given concentrations) plus the biocides Kathon CG/IPC (diluted 1:200), 0.2 g/L azide, and 0.3 g/L $HgCl_2$.

Effluent samples were taken every 20 minutes as previously described and analyzed immediately after collection for BTEX, MtBE, and/or ethanol using gas chromatography.

3.5.2. Natural Attenuation Profiles Along Aquifer Columns

Three additional (larger) columns (30-cm long, 2.5-cm diameter) were used to further investigate natural attenuation of BTEX and ethanol, and their potential interactive effects. Emphasis was placed on obtaining concentration profiles along the length of the columns to investigate geochemical transitions and spatial variations in removal efficiency. The columns were equipped with 6 sampling ports (at 3, 6, 10, 15, 20, and 25 cm from the inlet), and packed with aquifer material from the Travis AFB site, CA (Table 3-2). One column was amended with BTEX (i.e., benzene 5.2 mg/L, toluene 4.1 mg/L, ethylbenzene 2.3 mg/L, *m*+*p*-xylenes

2.4 mg/L, *o*-xylene 2.5 mg/L), to provide a baseline for the effect of MtBE or ethanol on BTEX attenuation. Another column was amended with BTEX plus ethanol (100 mg/L). The third column was a sterile control to distinguish biodegradation from potential abiotic losses. This column was poisoned with Kathon biocide (1.5 mg/L) and amended with BTEX plus ethanol. Each column was fed continuously in an upflow mode at ~3 mL/h using both a peristaltic pump (Masterflex Mod. 7519-15) to supply the mineral medium (Table 3-12) and a syringe pump (Harvard Apparatus Mod. 22) to supply the volatile organic compounds (i.e., BTEX and ethanol). The ratio of the peristaltic to syringe pump rates was set at 20:1. The flow rate was ~3 mL/h (superficial velocity of 0.61 cm/h), and approximately 2 days were required to displace one pore volume.

3.6. Analytical Methods

Aqueous samples (1-mL) were collected using gas-tight syringes and analyzed for BTEX, MtBE, and ethanol using a Hewlett Packard 5890 Series II gas chromatograph (GC) equipped with a HP 19395A headspace autosampler and flame-ionization and photoionization detectors in series. Separation was achieved using a J&W Scientific DB-WAX column at 35^{o}C. Detection limits were approximately 0.02 mg/L for each BTEX compound, 0.01 mg/L for MtBE and 0.15 mg/L for ethanol.

Acetate, nitrite, nitrate, and sulfate were analyzed with a Dionex 4500i ion chromatograph using an AS4A ion exchange column for separation followed by chemical suppression and conductivity detection. The samples were passed through a 0.20-µm filter prior to ion chromatography analysis.

Headspace samples (100 µL) were collected for methane analysis using gas-tight syringes. Samples were injected into a Hewlett Packard 5890 Series II GC equipped with a flame ionization detector. Separation was achieved using a J&W Scientific DB-WAX column isothermally at 35 ^{o}C.

Ferrous iron (Fe [II]) was measured by the ferrozine method (Lovley and Phillips, 1986). Briefly, a 0.1 mL aqueous sample was collected from the microcosms inside the anaerobic chamber. The sample was diluted 1:50 into 4.9 mL 0.5 N HCl and allowed to digest for 20 min prior to filtering through a nylon filter (Alltech Associates, Inc.; pore diameter 0.20 µm). A 0.1-mL subsample of the acid-digested filtrate was then added to 4.9 mL ferrozine in HEPES buffer and allowed to react for 20 min. Fe (II) was then quantified colorimetrically at 562 nm in a spectrophotometer. The Fe(II) data must be considered suspect because the 0.23 mM sulfate present in the medium could have been microbially reduced to hydrogen sulfide, which could have abiotically reduced ferric iron (Beller *et al.*, 1992). Therefore, we have not included the Fe(II) data because it may be unrepresentative. These data, however, can be found in the appendices.

3.7. Results and Discussion

3.7.1. Microcosm Degradation Assays

This section addresses how ethanol and MtBE affect BTEX degradation under different electron-accepting conditions found in contaminated aquifers experiencing natural attenuation. For the sake of clarity and brevity, only data for cases where biodegradation was unequivocally established are presented. The rest of the data are presented in the appropriate appendices.

3.7.1.1. Travis AFB Microcosms

These microcosms were prepared with aquifer material of known exposure history to BTEX and MtBE (Table 3-2), and exhibited a relatively high BTEX degradation activity compared to the microcosms from other sites tested. Significant BTEX and ethanol removal in viable microcosms relative to sterile controls provided evidence of biodegradation (Table 3-13). MtBE, however, was not degraded in this experiment.

Under aerobic conditions some BTEX compounds (i.e., toluene and ethylbenzene) were degraded earlier than ethanol (Figure 3-2B). This is contrary to previous reports that ethanol degrades preferentially and that its presence delays the start of BTEX degradation (Corseuil *et al.*, 1998). This discrepancy is probably related to differences in exposure history and initial composition of the microbial communities present in the tested aquifer materials. Previous exposure to BTEX might have resulted in a higher initial concentration of adapted BTEX degraders at this site, which would have made it less susceptible to inhibition by ethanol compared to the uncontaminated material used by Corseuil *et al.* (1998).

No anaerobic benzene degradation was observed in two months of incubation. Benzene is usually the most recalcitrant of the BTEX compounds under anaerobic conditions (Heider *et al.*, 1998). While benzene mineralization has been reported in nitrate-reducing (Burland and Edwards, 1999), ferric iron-reducing (Lovley *et al.*, 1996), sulfidogenic (Edwards and Grbić-Galić**,** 1992), and methanogenic microcosms (Grbić-Galić and Vogel, 1987), with lag periods often exceeding one year (Kazumi *et al.*, 1997), the ubiquity of anaerobic benzene degraders has not been established. Thus, while the recalcitrance of benzene in these experiments could be due to insufficient incubation time to allow biodegradation to proceed, it is also plausible that no anaerobic microorganisms capable of degrading benzene were present in the tested material.

Toluene was the only BTEX compound degraded under all electron-accepting conditions and substrate combinations tested (Figures 3-2 to 3-6), and its degradation was faster in microcosms with stronger electron acceptors (i.e., electron acceptors with higher reduction potentials, such as oxygen and nitrate) (Table 3-13). Aerobic microcosms without ethanol degraded 1.5 mg/L of toluene within 4 days (Figure 3-2A), whereas denitrifying microcosms degraded this amount within one week (Figure 3-3A). Iron (III)-amended microcosms were slower, removing the added toluene in 10 days (Figure 3-4A). Sulfate-reducing microcosms were even slower, taking about two weeks to complete the removal of toluene (Figure 3-5A). "Methanogenic" microcosms were the slowest, and took about seven weeks to remove the added toluene (Figure 3-6A).

The degradation of toluene apparently supported the cometabolic biotransformation of all xylene isomers under denitrifying conditions, since xylene consumption coincided with that of toluene and subsided after toluene was removed (Figure 3-3). Cometabolism of xylene by toluene degraders appears to be a common substrate interaction under denitrifying conditions (Alvarez and Vogel, 1995; Evans *et al.,* 1991, 1992; Hutchins, 1991; Jensen *et al.*, 1990).

Ethanol (60 to 80 mg/L) was always the first compound to be degraded under anaerobic conditions (Figures 3-3B, 3-4B, 3-5B, and 3-6B), and its presence slowed down the degradation of toluene in iron (III)-amended (Figure 3-4) and "methanogenic" microcosms (Figure 3-6). Ethanol also inhibited the degradation of *m*+*p*-xylenes in iron (III)-amended microcosms (Figure 3-4B) and of *o*-xylene under sulfate-reducing conditions (Figure 3-5B). These compounds were only degraded in microcosms amended with BTEX alone or with MtBE. Logistic constraints precluded us from determining whether a longer incubation time would be required to observe xylene degradation in microcosms with ethanol.

MtBE was not degraded in this experiment, and its presence did not significantly affect the BTEX degradation patterns described above (Figures 3-2C, 3-3C, 3-4C, 3-5C, and 3-6C). BTEX and ethanol degradation generally coincided with the removal of the appropriate electron acceptor or the appearance of their reduced forms [e.g., Fe(II) and methane] (Appendix A). However, these data must be considered suspect for reasons discussed in Sections 3.3.1.1 and 3.6**.** No electron balances were calculated in this study because the electron acceptor demand from the added compounds was overshadowed by the higher and more variable background demand of the sediments. However, the dependence of the degradation of certain BTEX compounds on the presence of specific electron acceptors is apparent in some cases (e.g., the dependence of ethylbenzene degradation on the presence of nitrate; Figure 3-3B).

3.7.1.2. Tracy Site Microcosms

These microcosms were prepared with uncontaminated aquifer material (Table 3-2), and exhibited relatively low BTEX degradation activity with a smaller range of compounds degraded than the Travis AFB microcosms. All BTEX compounds were degraded under aerobic conditions (Figure 3-7), which reflects the ubiquitous nature of aerobic BTEX degraders. Ethanol was degraded earlier (within one week) than all BTEX compounds in aerobic microcosms (Figure 3-7B), and its presence hindered BTEX degradation (Table 3-14). This supports the hypothesis that a lack of previous BTEX exposure might make an aquifer material more susceptible to inhibition by ethanol, possibly hindering the acclimatization process.

Ethanol was degraded relatively quickly (within two weeks) in all anaerobic microcosms (Figures 3-8B, 3-9B, 3-10A, and 3-11A). Toluene was the only BTEX compound to degrade under some anaerobic conditions (Appendix B), and no toluene degradation was observed in sulfate-reducing and "methanogenic" microcosms during the 75-day incubation period. The presence of ethanol slowed down toluene degradation in iron (III)-amended microcosms, since no toluene degradation was observed during 75 days in triplicate incubations with ethanol.

3.7.1.3. Sacramento Site Microcosms

These microcosms were prepared with aquifer material with a history of BTEX and MtBE contamination (Table 3-2). Nevertheless, it exhibited a relatively small range of BTEX compounds degraded since benzene and *o*-xylene were not degraded in aerobic microcosms

within the two-week incubation period. Furthermore, no anaerobic BTEX degradation was observed except for toluene under denitrifying conditions (Table 3-15).

Ethanol was slowly degraded in aerobic microcosms, and it had an inhibitory effect on BTEX degradation (Figure 3-12B). No BTEX degradation was observed in triplicate aerobic microcosms with ethanol (Appendix C). Nevertheless, the presence of ethanol enhanced the degradation of toluene under denitrifying conditions (Figure 3-13), and toluene was not fully degraded in replicate microcosms without ethanol.

The observed enhancement of toluene degradation by the presence of ethanol under denitrifying conditions represents a caveat against generalizations about the effect of fuel additives on BTEX degradation patterns. Indeed, the diversity of microbial consortia may preclude generalizations about substrate interactions involving BTEX degradation by mixed cultures (Alvarez and Vogel, 1991).

MtBE was not degraded under any condition tested in this experiment, and its presence did not significantly affect the previously described BTEX degradation patterns.

3.7.1.4. Northwest Terminal Site Microcosms

These microcosms were prepared with aquifer material that had experienced BTEX contamination and a subsequent spill of neat ethanol (Table 3-2). Aerobic BTEX degradation activity was relatively low (Table 3-16), and none of the BTEX compounds was completely removed within the two-week incubation period (Figure 3-16). Furthermore, none of the xylene isomers was degraded in these aerobic microcosms (Appendix D). Although ethanol was degraded preferentially over BTEX compounds (Figure 3-16B), its presence did not significantly inhibit aerobic BTEX degradation compared to replicates without ethanol (Figure 3-16A).

Ethanol was readily degraded (within one week) under all electron acceptor conditions tested (Figures 3-17B, 3-18A, 3-19B, and 3-20A). No BTEX compounds were degraded in iron (III)-amended or "methanogenic" microcosms (Appendix D). Similar to microcosms from the Sacramento site, ethanol had a stimulatory effect on toluene degradation under denitrifying conditions (Figure 3-17), and no toluene degradation was observed in microcosms without ethanol within one and one half months of incubation. This enhancement is hypothesized to be due to fortuitous growth of toluene degraders during ethanol degradation.

Toluene was also degraded under sulfate-reducing conditions, and its consumption coincided with that of *m*+*p*-xylenes (Figure 3-19). Cometabolism of xylenes with toluene has been reported under sulfate-reducing conditions in studies with pure bacterial cultures (Beller *et al.,* 1996). Benzene, ethylbenzene, and *o*-xylene were not degraded anaerobically within two months of incubation.

Although electron acceptors were added in excess to preclude confounding effects associated with their depletion, the high electron acceptor demand exerted by ethanol (exacerbated by the background demand of the sediments) caused the complete removal of nitrate and sulfate. This caused toluene degradation to stop under denitrifying conditions until more nitrate was added (Figure 3-17B), and appeared to extend the lag period before the onset of toluene degradation under sulfate-reducing conditions (Figure 3-19B). This observation demonstrates the dependence of hydrocarbon degradation on these electron acceptors. In more general terms, it illustrates that when a gasohol spill occurs, ethanol degradation is likely to contribute

significantly to the depletion of electron acceptors and nutrients that could otherwise be available for BTEX degradation.

3.7.2. Summary of Microcosm Degradation Assays

Aquifer microcosms were used to investigate the effect of ethanol on microbial degradation of BTEX and MtBE under electron-accepting conditions commonly found in sites experiencing natural attenuation. Response variability was also investigated by preparing microcosms with aquifer material from different sites with different exposure histories. Material from Travis AFB, which had previous BTEX and MtBE contamination, exhibited the highest BTEX degradation activity and catabolic range under both aerobic and anaerobic conditions. Nevertheless, previous BTEX exposure did not always result in high degradation activity, as was the case for microcosms prepared from the Sacramento site. These microcosms were the only ones that did not degrade benzene and *o*-xylene under aerobic conditions, and similar to microcosms with uncontaminated material from the Tracy site, also failed to degrade any BTEX compound under sulfate-reducing and "methanogenic" conditions.

Ethanol was the only compound degraded under all conditions tested (Figure 3-21), and it generally degraded before any of the BTEX compounds. Ethanol was degraded faster under all electron acceptor conditions tested in Travis and Northwest Terminal microcosms (Figures 3-21A and 3-21D). Tracy and Sacramento microcosms required more time to remove it (Figures 3-21B and 3-21C). Similar to BTEX, ethanol degradation was slowest in Sacramento microcosms (Figure 3-21C), even though Tracy microcosms were the "controls" with no history of contamination (Figure 3-21B). In general, ethanol was degraded fastest under denitrifying conditions, and the rates under aerobic conditions were similar.

The rate coefficients determined for ethanol degradation (0.02 to 2 day^{-1}) are markedly faster than that reported for a field site where ethanol was used as a co-solvent for extraction of free phase chlorinated solvents (i.e., 0.33 $year^{-1}$) (Sewell *et al.*, 2001). It is unclear whether this discrepancy is due to toxicity of the high ethanol concentrations at this site (> 10,000 mg/L) or to more favorable conditions for biodegradation provided for the microcosms (e.g., higher temperature and nutrient addition).

The effect of ethanol on BTEX degradation was variable and is best illustrated for toluene, which was the BTEX compound that was most frequently degraded. In some cases, ethanol retarded toluene degradation (e.g., in "methanogenic" and iron (III)-amended microcosms from Travis AFB, in iron (III)-amended microcosms from the Tracy site, and in aerobic microcosms from the Sacramento site), but it occasionally enhanced toluene degradation under denitrifying conditions (e.g., for the Northwest Terminal and Sacramento sites) (Table 3-17). Enhancement of toluene degradation in the presence of ethanol may be attributable to the fortuitous growth of BTEX-degrading bacteria during ethanol degradation. It should be kept in mind, however, that electron acceptors were added in excess in these experiments, to preclude confounding effects associated with their limitation. However, the high electron acceptor demand exerted by ethanol at gasohol-contaminated sites would contribute to the depletion of electron acceptors, which would hinder BTEX degradation. This was observed in denitrifying and sulfate-reducing microcosms from the Northwest Terminal site.

Benzene, which is the most toxic of the BTEX compounds, was not degraded under any anaerobic electron-accepting conditions tested in this work (i.e., denitrifying, iron (III)-amended,

sulfate-reducing, and "methanogenic"). Although the lack of anaerobic benzene degradation might be due to insufficient incubation time, these results reflect the relative recalcitrance of benzene under anaerobic conditions. This suggests that one of the most important detrimental effects of ethanol would be the depletion of oxygen and the induction of anaerobiosis, which would hinder benzene biodegradation.

MtBE was not degraded under any condition tested in this experiment, and its presence did not significantly affect BTEX degradation patterns. The recalcitrance of MtBE precluded the assessment of how ethanol might affect the natural attenuation (if any) of pre-existing MtBE contamination. Nevertheless, the fact that MtBE seems to be even more recalcitrant under anaerobic conditions suggests that competition for oxygen by ethanol-degrading bacteria would also hinder MtBE biodegradation.

3.8. Effect of Ethanol and MtBE on BTEX Retardation

The addition of oxygenates to gasoline could affect the equilibrium partitioning of BTEX compounds between aqueous, fuel, and solid phases (i.e., the cosolvency effect). Specifically, MtBE or ethanol could reduce the polarity of the aqueous phase allowing higher concentrations of moderately hydrophobic compounds (e.g., BTEX) in the aqueous phase (Heermann and Powers, 1998). In theory, the cosolvent effect exerted by either MtBE or ethanol could also enhance the mobility of dissolved BTEX compounds by decreasing sorption-related retardation. Such effects, however, are concentration-dependent, and were not observed when MtBE or ethanol was fed continuously to sterile aquifer columns (as a co-contaminant with BTEX) at 10,000 mg/L (Figure 3-22A). This is evident by the similarity of the breakthrough data from columns fed toluene alone or with ethanol or MtBE. Apparently, this oxygenate concentration is much lower than is required to create significant cosolvent effects (Heermann and Powers, 1998; Powers *et al.*, 2001). Since this ethanol concentration is unlikely to be exceeded in gasohol-contaminated sites, adding ethanol to gasoline (e.g., <15% v/v) should not have a significant impact on BTEX retardation factors. Nevertheless, neat spills of ethanol (e.g., at a bulk terminal) could result in very high ethanol concentrations in a localized area, exerting a significant co-solvent effect that could exacerbate groundwater pollution by mobilizing pre-existing petroleum product releases. This is illustrated in Figure 3-22B. At 50%, ethanol enhanced the migration of toluene, as well as of benzene and *o*-xylene (data not shown), which traveled unretarded at the same velocity as the bromide tracer.

3.9. Effect of Ethanol and MtBE on BTEX Breakthrough from Non-Sterile Columns

Breakthrough experiments were repeated under non-sterile conditions to incorporate biodegradation effects and determine how ethanol or MtBE might affect the natural attenuation of BTEX plumes. BTEX, ethanol, and MtBE were fed continuously at similar concentrations to those used in the microcosm experiments, but electron acceptors (e.g., O_2) were not supplied in excess (Table 3-12) to mimic limitations commonly encountered *in situ*.

When present as a co-substrate, ethanol hindered BTEX degradation and resulted in decreased BTEX attenuation relative to MtBE-amended or unamended control columns. This is evident by the fact that both the maximum BTEX concentrations that broke through and the

residual effluent concentrations after pseudo-steady-state was achieved were significantly lower in the columns without ethanol, as illustrated for benzene (Figure 3-23) and toluene (Figure 3-24). Breakthrough curves for other BTEX compounds are presented in Appendix F. Note that effluent BTEX concentrations were very similar for the poisoned control and the ethanol-amended column, indicating that no significant BTEX biodegradation occurred when ethanol was added.

Similar to the microcosm experiment, MtBE was not degraded (Figure 3-25) and its presence did not affect BTEX breakthrough patterns (Figures 3-23 and 3-24). Ethanol, on the other hand, was rapidly degraded (Figure 3-25). The degradation of ethanol was presumed to occur predominantly under anaerobic conditions because the oxygen demand exerted by the influent ethanol (313 mg/L as ultimate biological oxygen demand for 150 mg/L ethanol) far exceeded the available dissolved oxygen (ca. 9 mg/L). This suggests that oxygen depletion due to ethanol degradation near the column influent was at least partially responsible for the observed negative effect on BTEX degradation. To test this hypothesis and learn more about geochemical transitions and spatial variations in BTEX removal efficiency, three additional (larger) columns equipped with side-ports were operated. Results from this additional column experiment are discussed in the next section.

3.10. Natural Attenuation Profiles Along Aquifer Columns

No significant decreases in BTEX and ethanol (<5 %) were observed in the sterile control column, showing that volatile losses were relatively minor. BTEX were rapidly degraded within the first 10 cm of the column inlet when added without ethanol (Figure 3-26A).

BTEX degradation was adversely affected by the presence of ethanol, which was preferentially utilized within 3 cm of the column inlet (Figure 3-26B). These results corroborate the previous column study (Section 3.9) as well as previous reports that ethanol can be degraded faster than other gasoline constituents (Corseuil *et al.*, 1998). Ethanol concentrations are expected to exceed 1,000 mg/L near the source of a gasohol release (Powers *et al.*, 2001). Thus, ethanol is likely to exert a significant biochemical demand for oxygen and other electron acceptors (e.g., nitrate and sulfate) compared to other soluble gasoline components (Figure 3-27). Note that the mineral medium contained ammonium as the nitrogen source (Table 3-12), which precluded the use of nitrate as a nutrient. The high electron-acceptor demand exerted by ethanol created reducing conditions (-29 mV) near the column inlet (Figure 3-28), which reflects conditions that are typically not conducive to rapid BTEX degradation. The rapid depletion of oxygen during ethanol degradation is particularly important for the fate of benzene, which is the most toxic of the BTEX compounds and degrades slowly or not at all under anaerobic conditions (Alvarez and Vogel, 1995; Heider *et al.*, 1998).

Because of the high oxygen demand exerted by gasohol spills, ethanol is likely to be degraded predominantly under anaerobic conditions. None of the products of anaerobic ethanol degradation is toxic, although some metabolites such as butyrate (Gottschalk, 1986) could adversely affect the taste and odor of groundwater supplies. In addition, ethanol-derived acetate and other volatile fatty acids can cause a decrease in pH if they accumulate. In this study, acetate was detected (up to 200 mg/L) only in the column fed BTEX plus ethanol (Figure 3-29A). Acetate production caused a small decrease in pH, from about 7 to 6 pH units (Figure 3-29B).

Poorly buffered systems could experience a larger decrease in pH that could inhibit microbial activity and the further degradation of ethanol and other compounds.

Another potential concern is the accumulation of ethanol-derived methane, which could represent an explosion hazard. Stoichiometric considerations suggest that (at 15°C) a 1,000-mg/L ethanol concentration could produce up to 0.77 L of methane within a one-liter pore volume (Powers *et al.*, 2001). In these experiments, however, methane accumulation was not a major concern, possibly because of the addition of multiple electron acceptors that compete with methanogenesis. Methane was detected only in the column fed BTEX plus ethanol, and the highest observed concentration was 1.2 mg/L (Figure 3-30), which is considerably below its solubility (~24 mg/L at 20ºC). Nevertheless, some gasohol spills could pose a greater explosion risk when site-specific conditions favor more extensive methanogenesis and methane accumulation (e.g., higher ethanol concentrations and low nitrate and sulfate levels).

3.11. Conclusions

This study investigated the potential effects of ethanol and MtBE on the natural attenuation of BTEX compounds. On the basis of aquifer microcosm studies, the following conclusions were made:

- Ethanol is likely to be preferentially utilized relative to all of the BTEX compounds under aerobic and a range of anaerobic conditions. Therefore, while ethanol is unlikely to persist for extended periods of time at gasohol-contaminated sites, its presence may prevent the bacterial population capable of degrading BTEX from fully expressing its catabolic potential and would thus retard BTEX degradation.
- Toluene was degraded under all electron-accepting conditions tested, and its degradation rate generally increased with increasing reduction potential of electron acceptors (i.e., degradation under aerobic and denitrifying conditions tended to be faster than under other anaerobic conditions tested). Some other alkylbenzenes (ethylbenzene, xylenes) were also degraded anaerobically. Benzene, however, was only degraded in aerobic microcosms, and was recalcitrant under all anaerobic conditions tested. This suggests that oxygen depletion during ethanol biodegradation is likely to hinder the biodegradation of benzene to a greater extent than that of other BTEX compounds.
- If electron acceptors are available in excess, ethanol may exhibit a variable effect. While ethanol is more likely to hinder BTEX degradation, it may occasionally enhance anaerobic alkylbenzene degradation, possibly due to additional growth of BTEX-degrading bacteria during ethanol degradation. If electron acceptors are limiting, on the other hand, their depletion during ethanol degradation is likely to exacerbate the negative effect that ethanol may have on the metabolism of BTEX.
- MtBE was not degraded in this study, and the presence of this recalcitrant compound is not likely to affect BTEX degradation.

On the basis of flow-through aquifer column studies, the following conclusions were made:

- Cosolvent effects that increase the dissolution and migration of BTEX compounds are unlikely to occur at the ethanol or MtBE concentrations expected at reformulated

gasoline-contaminated sites (i.e., < 10,000 mg/L), but could be important when dealing with neat ethanol releases at bulk terminals.

- Ethanol constitutes a significant additional electron acceptor demand compared to that exerted by other soluble components of gasoline, and is likely to cause the depletion of the preferred nutrients and electron acceptors that could otherwise be available for BTEX degradation. A decrease in the extent of aerobic BTEX degradation in oxygen-limited aquifers is particularly important for the fate of benzene, which is the most toxic of the BTEX compounds and degrades very slowly, if at all, under anaerobic conditions.
- The preferential degradation of ethanol and the accompanying depletion of oxygen and other electron acceptors suggest that ethanol could hinder the natural attenuation of BTEX plumes. Nevertheless, it is unknown to what extent ethanol would increase the distance that benzene migrates before being attenuated to acceptable concentrations by natural processes. Therefore, field and modeling studies should be conducted to quantify the effect of ethanol on plume length and improve our risk assessment capabilities.

3.12. Recommendations

- A Controlled Release and Additional Study of Field Sites: These laboratory experiments suggest that ethanol is likely to hinder BTEX natural attenuation. One major concern is that ethanol could increase the distance that BTEX compounds migrate before being attenuated to acceptable concentrations by natural processes. Nevertheless, there is considerable uncertainty regarding the magnitude and significance of this potential impact. Therefore, field-scale and modeling studies should be conducted to quantify the effect of ethanol on plume length. Such studies could include controlled-release (field) experiments and statistical analyses of LUFT site data with and without ethanol.
- Better Integration of Ethanol Degradation Kinetics into Models: Mathematical fate and transport models should also be developed (and calibrated) to integrate the negative effects of ethanol on BTEX degradation (e.g., electron acceptor depletion) with potential positive effects (e.g., enhanced bacterial growth). Such models would be useful for risk assessment and management purposes.
- Improved Anaerobic Biostimulation Strategies: Longer BTEX plumes represent a greater risk of exposure to potential downgradient receptors, which could result in decreased acceptability of natural attenuation as a remedial approach at some sites. This could stimulate a shift of cleanup decisions towards engineered remediation approaches. Although the most common engineered bioremediation approaches used for BTEX cleanup are aerobic, introducing sufficient oxygen to meet the high oxygen demand exerted by ethanol will likely be technically difficult and prohibitively expensive. Therefore, anaerobic biostimulation strategies should be considered. However, the lack of field experience with enhanced anaerobic bioremediation approaches for BTEX contamination will require the refinement and demonstration of suitable approaches. These could include the addition of nitrate to increase the electron acceptor pool (in a manner that does not create toxicity or clogging problems), and bioaugmentation with anaerobic cultures that can degrade benzene, which is relatively recalcitrant under anaerobic conditions.

- Improved Characterization of Methane and Volatile Fatty Acids at Ethanol Release Sites: Neat ethanol spills and some gasohol releases could pose an explosion risk when site-specific conditions favor extensive methanogenesis and methane accumulation. In addition, ethanol-derived acetate and other volatile fatty acids could cause a decrease in pH (thus hindering biodegradation processes) and create taste and odor problems. Therefore, site characterization protocols should include methane and volatile fatty acid analyses near the source zone.

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Tables

Table 3-1. Characteristics of aquifer materials used in this work[a].

Component	Site			
	Travis[b]	Tracy[c]	Sacramento[d]	Northwest Terminal[e]
pH	6.9	7.3	7.4	5.7
Organic matter (%)	0.9	0.6	1.1	0.9
Salts electrical conductivity (mmhos/cm)	0.2	4.3	0.3	0.4
Cation exchange capacity (meq/100g)	4.2	5	13.6	9.3
P (ppm)	14	9	7	34
K (ppm)	70	10	70	130
Ca (ppm)	400	500	1,700	1,300
Mg (ppm)	240	300	600	300
Na (ppm)	70	17	13	7
S (ppm)	30	81	4	7
Zn (ppm)	0.8	0.7	1.2	1.1
Cu (ppm)	2.4	0.6	1.2	1.2
Mn (ppm)	70	16	70	111
Fe (ppm)	109	38	80	171
B (ppm)	0.6	NR	NR	NR

[a] Minnesota Valley Testing Laboratories, Inc. performed analysis.

[b] Travis Air Force Base, BTEX and MtBE exposure history.

[c] Tracy (CA), no previous BTEX exposure.

[d] Northwest Terminal site, ethanol (average 16,100 mg/L) and BTEX exposure history.

[e] Sacramento (CA), BTEX and MtBE exposure history.

NR = Not reported.

Table 3-2. Key constituent concentrations in groundwater at the sample collection point.

	Site			
Component	**Travis[a]**	**Tracy[b]**	**Sacramento[c]**	**Northwest Terminal[d]**
Benzene (ppb)	**300**	**ND**	**5,300**	**1,500**
Toluene (ppb)	**170**	**ND**	**35**	**6,090**
Ethylbenzene (ppb)	**4,700**	**ND**	**270**	**768**
Xylenes (ppb)	**3,100**	**ND**	**130**	**5,070**
Ethanol (ppm)	**NR**	**ND**	**NA**	**16,100**
MtBE (ppb)	**~170**	**ND**	**2,700**	**ND**
Nitrate (ppm)	**ND**	**NA**	**ND[e]**	**ND**
Sulfate (ppm)	**27**	**NA**	**13**	**307**
DO (ppm)	**1 to 2**	**NA**	**NA**	**ND**

[a] **Travis Air Force Base, BTEX and MtBE exposure history.**

[b] **Tracy (CA), no previous BTEX exposure.**

[c] **Northwest Terminal site, ethanol and BTEX exposure history.**

[d] **Sacramento (CA), BTEX and MtBE exposure history.**

[e] **0.02-mM detection limit.**

NR = Not reported. ND = Not detected. NA = Data not available.

Table 3-3. Initial BTEX, ethanol, and MtBE concentrations added to Travis AFB microcosms.

Microcosm set	Benzene (mg/L)	Toluene (mg/L)	Ethyl-benzene (mg/L)	*o*-Xylene (mg/L)	*m*+*p*-xylenes (mg/L)	MtBE (mg/L)	Ethanol (mg/L)
Aerobic							
1	1.7±0.2	1.2±0.1	0.3±0.5	0.5±0.1	0.6±0.1	NA	NA
2	1.6±0.1	1.2±0.1	0.3±0.1	0.6±0.0	0.5±0.0	NA	60.5±0.3
3	1.6±0.1	1.2±0.1	0.3±0.0	0.5±0.0	0.5±0.0	5.4±0.3	NA
4	1.6±0.1	1.2±0.0	0.3±0.0	0.5±0.0	0.5±0.0	5.6±0.2	60.3±1.6
Abiotic controls	1.7±0.0	1.3±0.1	1.4±0.0	0.5±0.0	0.6±0.0	5.8±0.0	58.9±0.7
Denitrifying							
5	1.8±0.2	1.5±0.2	0.4±0.0	0.5±0.1	0.5±0.1	NA	NA
6	1.9±0.1	1.8±0.2	0.4±0.0	0.6±0.0	0.6±0.0	NA	54.4±4.9
7	1.7±0.1	1.3±0.1	0.3±0.0	0.5±0.1	0.4±0.0	6.3±0.2	NA
Iron (III)-amended							
8	1.8±0.2	1.6±0.2	0.4±0.1	0.6±0.1	0.6±0.1	NA	NA
9	1.9±0.2	1.6±0.2	0.4±0.1	0.6±0.1	0.6±0.1	NA	54.3±3.8
10	1.7±0.0	1.3±0.1	0.3±0.1	0.5±0.1	0.4±0.1	6.0±0.2	NA
Sulfate-reducing							
11	1.7±0.1	1.4±0.0	0.4±0.0	0.5±0.0	0.5±0.1	NA	NA
12	1.8±0.2	1.5±0.2	0.4±0.0	0.5±0.1	0.5±0.1	NA	56.0±2.5
13	1.8±0.1	1.5±0.2	0.3±0.0	0.5±0.1	0.5±0.1	6.4±0.3	NA
"Methanogenic" microcosms							
14	1.9±0.1	1.5±0.1	0.4±0.2	0.6±0.3	0.6±0.3	NA	NA
15	1.8±0.0	1.5±0.2	0.4±0.0	0.5±0.0	0.5±0.0	NA	50.8±4.7
16	2.1±0.9	1.7±0.7	0.4±0.2	0.6±0.3	0.6±0.3	6.8±1.3	NA
Abiotic (anaerobic) controls							
17	1.4±0.2	1.1±0.2	0.3±0.1	0.4±0.1	0.4±0.1	10.2±0.5	52.9±2.3

NA = Compound was not added. Each set consisted of three microcosms, and values represent mean±SD.

Table 3-4. Electron acceptor amendments for Travis AFB microcosms.

Set	Soil (g)	Medium (mL)	Electron acceptor
1	20	80	O_2 (pure O_2 flushed through headspace)
2	20	80	O_2 (pure O_2 flushed through headspace)
3	20	80	O_2 (pure O_2 flushed through headspace)
4	20	80	O_2 (pure O_2 flushed through headspace)
	20	80	Aerobic control (O_2 flushed through headspace)
5	20	80	NO_3^- (322 mg/L)
6	20	80	NO_3^- (333 mg/L)
7	20	80	NO_3^- (333 mg/L)
8	20	80	Fe (III) (845 mg/L ferric oxyhydroxide)
9	20	80	Fe (III) (845 mg/L ferric oxyhydroxide)
10	20	80	Fe (III) (845 mg/L ferric oxyhydroxide)
11	20	80	SO_4^{2-} (388 mg/L)
12	20	80	SO_4^{2-} (383 mg/L)
13	20	80	SO_4^{2-} (383 mg/L)
14	20	80	CO_2 (454 mg/L as HCO_3^-)
15	20	80	CO_2 (454 mg/L as HCO_3^-)
16	20	80	CO_2 (454 mg/L as HCO_3^-)
17	20	80	Anaerobic control, NO_3^- (934 mg/L), Fe (III) (845 mg/L ferric oxyhydroxide), SO_4^{2-} (312 mg/L)

Table 3-5. Initial BTEX and ethanol concentrations added to Tracy site microcosms.

Microcosm set	Benzene (mg/L)	Toluene (mg/L)	Ethyl-benzene (mg/L)	*o*-Xylene (mg/L)	*m*+*p*-xylenes (mg/L)	Ethanol (mg/L)
Aerobic conditions						
1	1.9±0.2	1.4±0.2	0.4±0.1	0.5±0.1	0.6±0.1	NA
2	1.3±0.3	0.9±0.2	0.3±0.1	0.4±.0.1	0.4±0.1	58.4±3.6
Abiotic controls	1.5±0.0	1.1±0.0	0.3±0.0	0.4±0.0	0.5±0.0	55.9±2.6
Nitrate microcosms						
3	2.5±0.1	1.6±0.1	0.5±0.0	0.7±0.0	0.7±0.0	NA
4	2.5±0.1	1.5±0.1	0.5±0.0	0.7±0.0	0.6±0.0	71.9±6.5
Iron (III)-amended microcosms						
5	2.8±0.2	1.7±0.1	0.5±0.0	0.8±0.1	0.7±0.0	NA
6	2.6±0.0	1.6±0.0	0.5±0.0	0.7±0.0	0.7±0.0	85.4±6.6
Sulfate microcosms						
7	2.6±0.1	1.6±0.0	0.5±0.0	0.7±0.0	0.7±0.0	NA
8	2.6±0.1	1.6±0.1	0.5±0.0	0.7±0.0	0.7±0.0	79.7±2.6
"Methanogenic" microcosms						
9	2.6±0.1	1.6±0.1	0.5±0.0	0.7±0.0	0.7±0.0	NA
10	2.5±0.1	1.5±0.1	0.5±0.0	0.7±0.0	0.6±0.0	81.0±7.8
Abiotic controls						
11	2.2±0.5	1.3±0.4	0.4±0.1	0.6±0.2	0.6±0.2	50.2±22.7

NA = Compound was not added. Each set consisted of three microcosms, and values represent mean±SD.

Table 3-6. Electron acceptor amendments for Tracy site microcosms.

Set	Soil (g)	Medium (mL)	Electron acceptor
1	20	80	O_2 (pure O_2 flushed through headspace)
2	20	80	O_2 (pure O_2 flushed through headspace)
	20	80	Aerobic control (O_2 flushed through headspace)
3	20	80	NO_3^- (337 mg/L)
4	20	80	NO_3^- (387 mg/L)
5	20	80	Fe (III) (845 mg/L ferric oxyhydroxide)
6	20	80	Fe (III) (845 mg/L ferric oxyhydroxide)
7	20	80	SO_4^{2-} (531 mg/L)
8	20	80	SO_4^{2-} (521 mg/L)
9	20	80	CO_2 (454 mg/L as HCO_3^-)
10	20	80	CO_2 (454 mg/L as HCO_3^-)
11	20	80	Anaerobic control, NO_3^- (705 mg/L), Fe (III) (845 mg/L ferric oxyhydroxide), SO_4^{2-} (470 mg/L)

Table 3-7. Initial BTEX, ethanol, and MtBE concentrations added to Sacramento site microcosms.

Microcosm Set	Benzene (mg/L)	Toluene (mg/L)	Ethyl-benzene (mg/L)	*o*-Xylene (mg/L)	*m+p*-xylenes (mg/L)	MtBE (mg/L)	Ethanol (mg/L)
Aerobic microcosms							
1	2.0±0.0	1.5±0.1	0.5±0.0	0.7±0.0	0.8±0.0	NA	NA
2	2.1±0.1	1.6±0.1	0.5±0.0	0.8±0.0	0.8±0.1	NA	72.6±1.0
3	2.1±0.1	1.6±0.1	0.5±0.0	0.8±0.0	0.8±0.0	7.3±0.2	NA
4	2.2±0.1	1.6±0.1	0.5±0.0	0.8±0.0	0.8±0.0	7.5±0.3	72.2±0.7
Abiotic controls	2.0±0.1	1.5±0.1	0.5±0.0	0.8±0.1	0.7±0.0	7.1±0.0	70.8±1.9
Nitrate microcosms							
5	2.6±0.1	1.7±0.1	0.5±0.0	0.7±0.0	0.8±0.0	NA	NA
6	2.4±0.0	1.6±0.0	0.5±0.0	0.7±0.0	0.7±0.0	NA	69.4±2.1
7	2.5±0.1	1.7±0.1	0.5±0.1	0.7±0.1	0.8±0.1	7.0±0.2	NA
Sulfate microcosms							
8	2.5±0.0	1.7±0.0	0.5±0.1	0.7±0.1	0.8±0.1	NA	NA
9	2.5±0.1	1.7±0.1	0.5±0.0	0.8±0.1	0.7±0.0	NA	70.1±2.0
10	2.5±0.1	1.7±0.1	0.5±0.0	0.7±0.0	0.8±0.1	7.1±0.1	NA
"Methanogenic" microcosms							
11	2.2±0.1	1.4±0.1	0.4±0.0	0.6±0.1	0.7±0.1	NA	NA
12	2.1±0.1	1.3±0.1	0.4±0.0	0.6±0.1	0.6±0.1	NA	75.9±1.8
13	2.0±0.5	1.3±0.3	0.4±0.1	0.5±0.1	0.6±0.1	5.9±1.5	NA
Abiotic controls							
14	2.0±0.3	1.2±0.2	0.4±0.1	0.5±0.1	0.6±0.1	5.6±0.9	70.3±6.9

NA = Compound was not added. Each set consisted of three microcosms, and values represent mean±SD.

Table 3-8. Electron acceptor amendments for Sacramento site microcosms.

Set	Soil (g)	Medium (mL)	Electron acceptor
1	20	80	O_2 (pure O_2 flushed through headspace)
2	20	80	O_2 (pure O_2 flushed through headspace)
3	20	80	O_2 (pure O_2 flushed through headspace)
4	20	80	O_2 (pure O_2 flushed through headspace)
	20	80	Aerobic control (O_2 flushed through headspace)
5	20	80	NO_3^- (363 mg/L)
6	20	80	NO_3^- (351 mg/L)
7	20	80	NO_3^- (336 mg/L)
8	20	80	SO_4^{2-} (397 mg/L)
9	20	80	SO_4^{2-} (385 mg/L)
10	20	80	SO_4^{2-} (377 mg/L)
11	20	80	CO_2 (454 mg/L as HCO_3^-)
12	20	80	CO_2 (454 mg/L as HCO_3^-)
13	20	80	CO_2 (454 mg/L as HCO_3^-)
14	20	80	Anaerobic control, NO_3^- (627 mg/L), SO_4^{2-} (393 mg/L)

Table 3-9. Initial BTEX and ethanol concentrations added to Northwest Terminal site microcosms.

Microcosm Set	Benzene (mg/L)	Toluene (mg/L)	Ethyl-benzene (mg/L)	*o*-Xylene (mg/L)	*m*+*p*-xylenes (mg/L)	Ethanol (mg/L)
Aerobic conditions						
1	2.1±0.0	1.5±0.0	0.5±0.0	0.7±0.0	0.8±0.0	NA
2	2.1±0.1	1.6±0.1	0.5±0.0	0.7±0.0	0.8±0.0	92.0±0.5
Abiotic controls	2.0±0.0	1.8±0.0	0.6±0.0	0.8±0.0	1.0±0.0	95.8±0.0
Nitrate microcosms						
3	2.6±0.1	1.5±0.0	0.4±0.0	0.6±0.0	0.6±0.0	NA
4	2.8±0.0	1.6±0.0	0.5±0.0	0.7±0.0	0.7±0.0	62.7±4.3
Iron (III)-amended microcosms						
5	2.5±0.1	1.5±0.1	0.5±0.0	0.6±0.0	0.6±0.0	NA
6	2.4±0.2	1.4±0.2	0.4±0.1	0.6±0.1	0.6±0.1	60.0±11.6
Sulfate microcosms						
7	2.5±0.2	1.5±0.1	0.5±0.0	0.6±0.0	0.6±0.0	NA
8	2.6±0.1	1.6±0.1	0.5±0.0	0.7±0.0	0.7±0.1	68.2±0.9
"Methanogenic" microcosms						
9	2.4±0.2	1.4±0.1	0.4±0.0	0.6±0.0	0.6±0.0	NA
10	2.4±0.0	1.4±0.0	0.4±0.0	0.6±0.0	0.6±0.0	72.9±2.3
Abiotic controls						
11	2.5±0.1	1.4±0.1	0.4±0.1	0.6±0.1	0.6±0.1	67.7±1.5

NA = Compound was not added. Each set consisted of three microcosms, and values represent mean±SD.

Table 3-10. Electron acceptor amendments for Northwest Terminal site microcosms.

Set	Soil (g)	Medium (mL)	Electron acceptor
1	20	80	O_2 (pure O_2 flushed through headspace)
2	20	80	O_2 (pure O_2 flushed through headspace)
	20	80	Aerobic control (O_2 flushed through headspace)
3	20	80	NO_3^- (373 mg/L)
4	20	80	NO_3^- (332 mg/L)
5	20	80	Fe (III) (845 mg/L ferric oxyhydroxide)
6	20	80	Fe (III) (845 mg/L ferric oxyhydroxide)
7	20	80	SO_4^{2-} (423 mg/L)
8	20	80	SO_4^{2-} (372 mg/L)
9	20	80	CO_2 (454 mg/L as HCO_3^-)
10	20	80	CO_2 (454 mg/L as HCO_3^-)
11	20	80	Anaerobic control, NO_3^- (584 mg/L), Fe (III) (845 mg/L ferric oxyhydroxide), SO_4^{2-} (310 mg/L)

Table 3-11. Experimental matrix for sacrificial microcosms used for each aquifer material.

Set	Soil (g)	Medium (mL)	Electron acceptor	BTEX	Ethanol
1	10	40	O_2	+	-
2	10	40	O_2	+	+
3	10	40	NO_3^-	+	-
4	10	40	NO_3^-	+	+
5	10	40	Fe (III)	+	-
6	10	40	Fe (III)	+	+
7	10	40	SO_4^{2-}	+	-
8	10	40	SO_4^{2-}	+	+
9	10	40	CO_2	+	-
10	10	40	CO_2	+	+
11*	10	40	CO_2	-	-

* Used to characterize initial conditions.

Compounds added are denoted by a positive sign (+) and their absence by a negative sign (-).

Table 3-12. Composition of mineral medium used in column experiments.

Compound	Concentration (mg/L)
K_2SO_4	40.1
$NaNO_3$	22.5
$CaCl_2$	111.0
$MgCl_2.6H_2O$	12.2
K_2HPO_4	0.1
$NaHCO_3$	201.6
H_3BO_3	3.71×10^{-4}
$Ni(NO_3)_2.6H_2O$	1.74×10^{-3}
$CuSO_4.5H_2O$	1.50×10^{-3}
$ZnSO_4.7H_2O$	1.73×10^{-3}
$CoSO_4.7H_2O$	1.69×10^{-3}
$(NH_4)_6Mo_7O_{24}.4H_2O$[a]	1.06×10^{-3}

[a] Ammonium molybdate (EM Science).

Table 3-13. Summary of BTEX and ethanol degradation patterns in Travis AFB microcosms. Numbers in parentheses are correlation coefficients for the rate coefficient determination.

	Aerobic		Denitrifying		Iron (III)-amended		Sulfate-reducing		"Methanogenic"	
Compound	Lag phase (Days)	Rate coefficient[a] (Day^{-1})	Lag phase (Days)	Rate coefficient[a] (Day^{-1})	Lag phase (Days)	Rate coefficient[a] (Day^{-1})	Lag phase (Days)	Rate coefficient[a] (Day^{-1})	Lag phase (Days)	Rate coefficient[a] (Day^{-1})
Benzene										
with BTEX	0	3.03(0.95)	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+EtOH	0	0.47(0.89)	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+MtBE	0	3.04(0.96)	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+EtOH+MtBE	0	0.19(0.98)	NA	NA	NA	NA	NA	NA	NA	NA
Toluene										
with BTEX	0	0.79(0.77)	2	0.37(0.87)	0	0.09(0.69)	6	0.31(0.93)	0	0.03(0.79)
with BTEX+EtOH	0	0.89(0.90)	0	0.19(0.89)	0	0.02(0.87)	3	0.11(0.75)	0	0.02(0.87)
with BTEX+MtBE	0	0.74(0.90)	2	0.45(0.86)	0	0.09(0.74)	6	0.23(0.83)	0	0.04(0.79)
with BTEX+EtOH+MtBE	0	0.59(0.90)	NA	NA	NA	NA	NA	NA	NA	NA
Ethylbenzene										
with BTEX	0	2.91(1.00)	2	0.27(0.88)	ND	ND	ND	ND	ND	ND
with BTEX+EtOH	0	4.85(1.00)	3	0.08(0.58)*	ND	ND	ND	ND	ND	ND
with BTEX+MtBE	0	4.04(1.00)	2	0.22(0.71)	ND	ND	ND	ND	ND	ND
with BTEX+EtOH+MtBE	0	4.31(1.00)	NA	NA	NA	NA	NA	NA	NA	NA
m+p - Xylenes										
with BTEX	0	0.90(0.85)	2	0.13(0.84)*	13	0.13(0.88)	6	0.11(0.81)	ND	ND
with BTEX+EtOH	0	0.29(0.84)	0	0.10(0.86)*	ND	ND	3	0.07(0.90)*	ND	ND
with BTEX+MtBE	0	0.80(0.90)	2	0.11(0.79)*	16	0.14(0.89)	9	0.11(0.90)	ND	ND
with BTEX+EtOH+MtBE	0	0.14(0.85)	NA	NA	NA	NA	NA	NA	NA	NA
o - Xylene										
with BTEX	0	1.16(0.88)	3	0.08(0.82)*	ND	ND	27	0.21(1.00)	ND	ND
with BTEX+EtOH	6	1.50(1.00)	3	0.02(0.90)	ND	ND	ND	ND	ND	ND
with BTEX+MtBE	0	1.36(0.82)	3	0.05(0.68)	ND	ND	27	0.11(1.00)	ND	ND
with BTEX+EtOH+MtBE	ND	ND	NA	NA	NA	NA	NA	NA	NA	NA
Ethanol										
with BTEX+EtOH	0	0.50(0.99)	0	1.28(0.78)	0	0.81(0.58)	0	0.09(0.89)	0	0.47(0.93)
with BTEX+EtOH+MtBE	0	0.23(0.99)	NA	NA	NA	NA	NA	NA	NA	NA
MtBE										
with BTEX+MtBE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+EtOH+MtBE	ND	ND	NA	NA	NA	NA	NA	NA	NA	NA

[a] Rate coefficients were obtained by fitting an exponential decay model to data after the lag period, and were corrected for abiotic losses by subtracting the corresponding coefficient from sterile controls (Appendix F). All sets were prepared in triplicate.

* Degradation ceased after toluene and ethylbenzene were removed.

ND = No significant removal relative to sterile control was observed. NA = Not available (i.e., microcosms were not prepared for this treatment).

Table 3-14. Summary of toluene and ethanol degradation patterns in Tracy site microcosms. Numbers in parentheses are correlation coefficients for the rate coefficient determination.

	Aerobic		Denitrifying		Iron (III)-amended		Sulfate-reducing		"Methanogenic"	
Compound	Lag phase (Days)	Rate coefficient[a] (Day^{-1})	Lag phase (Days)	Rate coefficient[a] (Day^{-1})	Lag phase (Days)	Rate coefficient[a] (Day^{-1})	Lag phase (Days)	Rate coefficient[a] (Day^{-1})	Lag phase (Days)	Rate coefficient[a] (Day^{-1})
Benzene										
with BTEX	7	1.47(0.78)	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+EtOH	3	0.28(0.97)	ND	ND	ND	ND	ND	ND	ND	ND
Toluene										
with BTEX	7	1.53(0.76)	29	0.07(0.87)	15	0.04(0.96)	ND	ND	ND	ND
with BTEX+EtOH	3	0.25(0.97)	21	0.05(0.89)	ND	ND	ND	ND	ND	ND
Ethylbenzene										
with BTEX	7	1.25(0.78)	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+EtOH	3	0.17(0.94)	ND	ND	ND	ND	ND	ND	ND	ND
m+p - Xylenes										
with BTEX	7	1.12(0.76)	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+EtOH	3	0.10(0.97)	ND	ND	ND	ND	ND	ND	ND	ND
o - Xylene										
with BTEX	7	0.66(0.84)	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+EtOH	5	0.11(0.95)	ND	ND	ND	ND	ND	ND	ND	ND
Ethanol										
with BTEX+EtOH	2	0.96(0.97)	1	0.83(0.86)	6	0.31(0.95)	6	0.80(0.96)	7	1.50(1.00)

[a] Rate coefficients were obtained by fitting an exponential decay model to data after the lag period, and were corrected for abiotic losses by subtracting the corresponding coefficient from sterile controls. All sets were prepared in triplicate.

ND = No significant removal relative to sterile control was observed.

Table 3-15. Summary of BTEX and ethanol degradation patterns in Sacramento site microcosms. Numbers in parentheses are correlation coefficients for the rate coefficient determination.

	Aerobic		Denitrifying		Sulfate-reducing		"Methanogenic"	
Compound	Lag phase (Days)	Rate coefficient[a] (Day^{-1})	Lag phase (Days)	Rate coefficient[a] (Day^{-1})	Lag phase (Days)	Rate coefficient[a] (Day^{-1})	Lag phase (Days)	Rate coefficient[a] (Day^{-1})
Benzene								
with BTEX	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+EtOH	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+MtBE	ND	ND	ND	ND	ND	ND	ND	ND
Toluene								
with BTEX	0	0.11(0.89)	ND	ND	ND	ND	ND	ND
with BTEX+EtOH	ND	ND	0	0.02(0.69)*	ND	ND	ND	ND
with BTEX+MtBE	0	0.07(0.85)	ND	ND	ND	ND	ND	ND
Ethylbenzene								
with BTEX	0	0.16(0.86)	ND	ND	ND	ND	ND	ND
with BTEX+EtOH	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+MtBE	0	0.10(0.83)	ND	ND	ND	ND	ND	ND
m+p - Xylenes								
with BTEX	0	0.56(0.93)	ND	ND	ND	ND	ND	ND
with BTEX+EtOH	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+MtBE	0	0.25(0.86)	ND	ND	ND	ND	ND	ND
o - Xylene								
with BTEX	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+EtOH	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+MtBE	ND	ND	ND	ND	ND	ND	ND	ND
Ethanol								
with BTEX+EtOH	0	0.02(0.91)	0	2.03(0.89)	23	0.40(0.88)	21	0.14(0.88)
MTbe								
with BTEX+MtBE	ND	ND	ND	ND	ND	ND	ND	ND

[a] Rate coefficients were obtained by fitting an exponential decay model to data after the lag period, and were corrected for abiotic losses by subtracting the corresponding coefficient from sterile controls. All sets were prepared in triplicate.

* Degradation ceased after ethanol was removed.

ND = No significant removal relative to sterile control was observed.

Table 3-16. Summary of BTEX and ethanol degradation patterns in Northwest Terminal site microcosms. Numbers in parentheses are correlation coefficients for the rate coefficient determination.

	Aerobic		Denitrifying		Iron (III)-amended		Sulfate-reducing		"Methanogenic"	
Compound	Lag phase (Days)	Rate coefficient[a] (Day^{-1})	Lag phase (Days)	Rate coefficient[a] (Day^{-1})	Lag phase (Days)	Rate coefficient[a] (Day^{-1})	Lag phase (Days)	Rate coefficient[a] (Day^{-1})	Lag phase (Days)	Rate coefficient[a] (Day^{-1})
Benzene										
with BTEX	8	0.16(0.95)	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+EtOH	11	0.12(1.00)	ND	ND	ND	ND	ND	ND	ND	ND
Toluene										
with BTEX	4	0.12(0.97)	ND	ND	ND	ND	17	0.18(0.97)	ND	ND
with BTEX+EtOH	10	0.14(0.92)	13	0.38(0.85)	ND	ND	27	0.27(0.94)	ND	ND
Ethylbenzene										
with BTEX	5	0.16(0.99)	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+EtOH	10	0.37(0.78)	ND	ND	ND	ND	ND	ND	ND	ND
m+p - Xylenes										
with BTEX	ND	ND	ND	ND	ND	ND	17	0.07(0.98)	ND	ND
with BTEX+EtOH	ND	ND	ND	ND	ND	ND	27	0.08(0.97)*	ND	ND
o - Xylene										
with BTEX	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
with BTEX+EtOH	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ethanol										
with BTEX+EtOH	2	0.63(0.80)	0	0.69(0.89)	0	0.37(0.59)	0	0.79(0.78)	0	0.35(0.59)

[a] Rate coefficients were obtained by fitting an exponential decay model to data after the lag period, and were corrected for abiotic losses by subtracting the corresponding coefficient from sterile controls. All sets were prepared in triplicate.

* Degradation ceased after toluene was removed.

ND = No significant removal relative to sterile control was observed.

Table 3-17. Time (days) required for a 50% decrease in toluene concentration in microcosms with material from different sites under different electron-accepting conditions.

	Site			
Conditions	**Travis[a]**	**Tracy[b]**	**Northwest Terminal[c]**	**Sacramento[d]**
Aerobic				
BTEX alone	**1**	**8**	**11**	**4**
with ethanol	**1**	**6**	**14**	**ND**
with MtBE	**1**	**NA**	**NA**	**7**
Denitrifying				
BTEX alone	**4**	**34**	**ND**	**ND**
with ethanol	**4**	**31**	**14**	**31**
with MtBE	**4**	**NA**	**NA**	**ND**
Iron (III)-amended				
BTEX alone	**5**	**29**	**ND**	**NA**
with ethanol	**18**	**ND**	**ND**	**NA**
with MtBE	**5**	**NA**	**NA**	**NA**
Sulfate-reducing				
BTEX alone	**11**	**ND**	**24**	**ND**
with ethanol	**7**	**ND**	**33**	**ND**
with MtBE	**11**	**NA**	**NA**	**ND**
"Methanogenic"				
BTEX alone	**5**	**ND**	**ND**	**ND**
with ethanol	**34**	**ND**	**ND**	**ND**
with MtBE	**6**	**NA**	**NA**	**ND**

[a] **Travis AFB, BTEX and MtBE exposure history.**

[b] **Tracy (CA), no previous BTEX exposure.**

[c] **Northwest Terminal site, ethanol (average 16, 100 mg/L) and BTEX exposure history.**

[d] **Sacramento (CA), BTEX and MtBE exposure history.**

NA = Data not available (i.e., not tested).

ND = No degradation was observed within the incubation period.

Figures

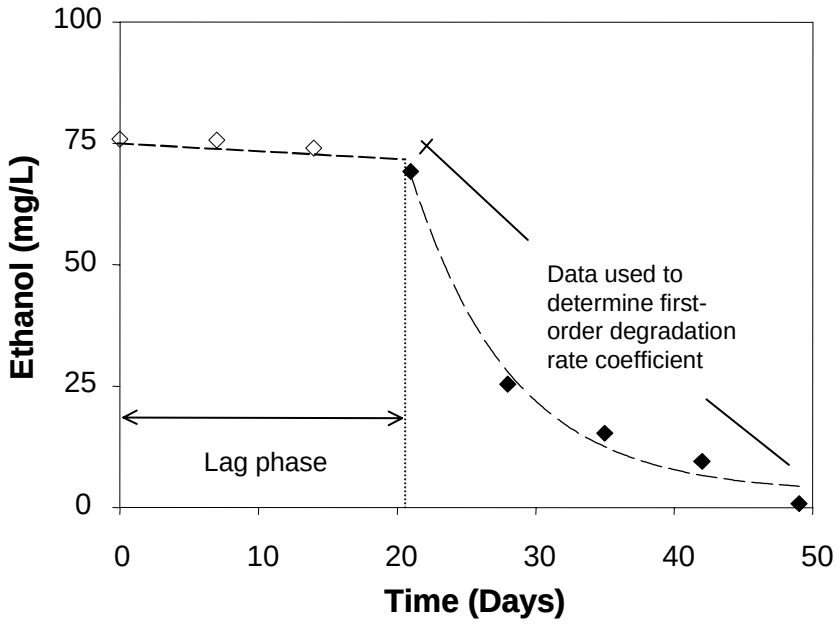


Figure 3-1. Example of procedure used to characterize degradation patterns in microcosms. These data represent average values from three replicates, and correspond to ethanol in "methanogenic" microcosms from the Sacramento site.

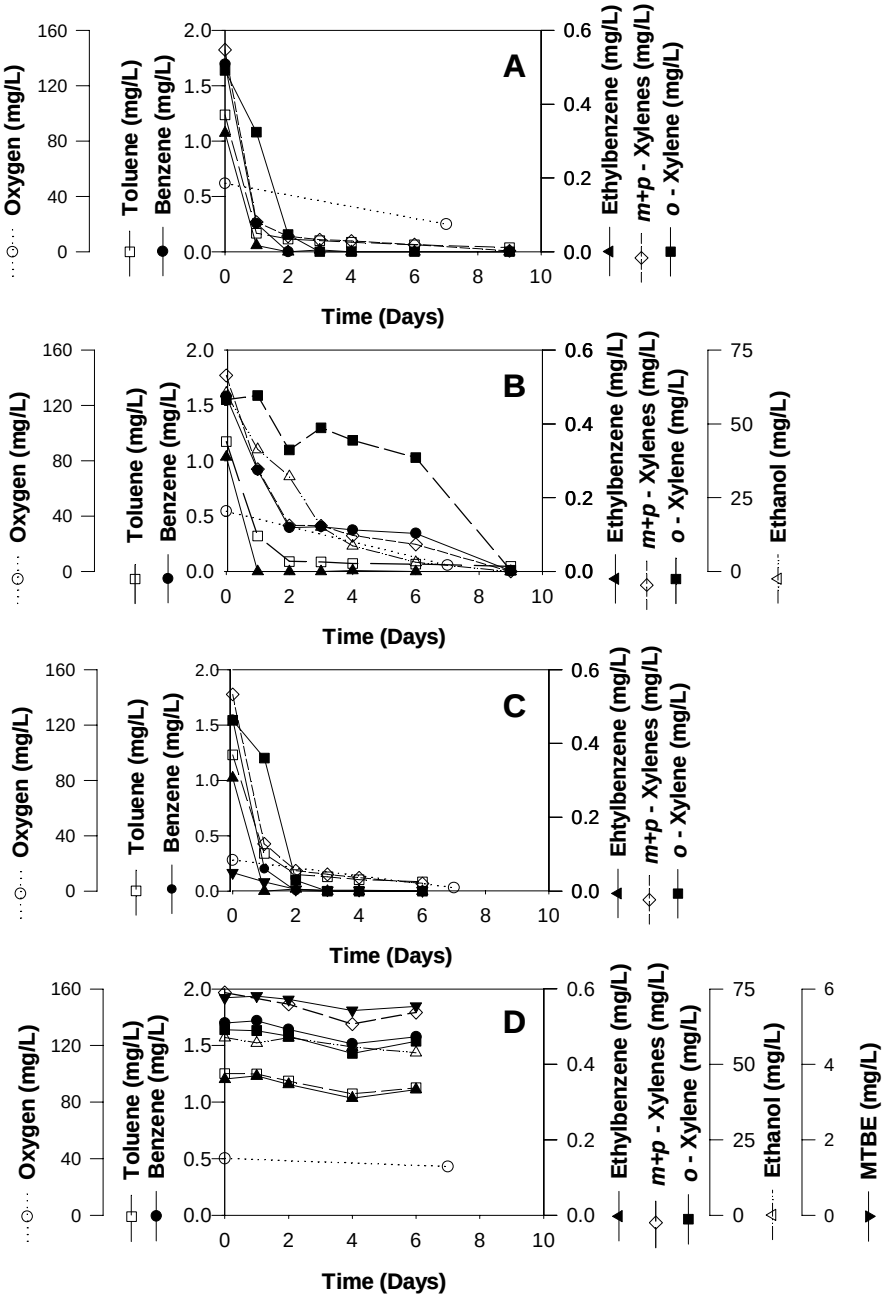


Figure 3-2. Degradation of BTEX and ethanol in aerobic microcosms from Travis AFB amended with BTEX alone (A); BTEX plus ethanol (B); BTEX plus MtBE (C); and Control (D). Data points correspond to average values from three replicates.

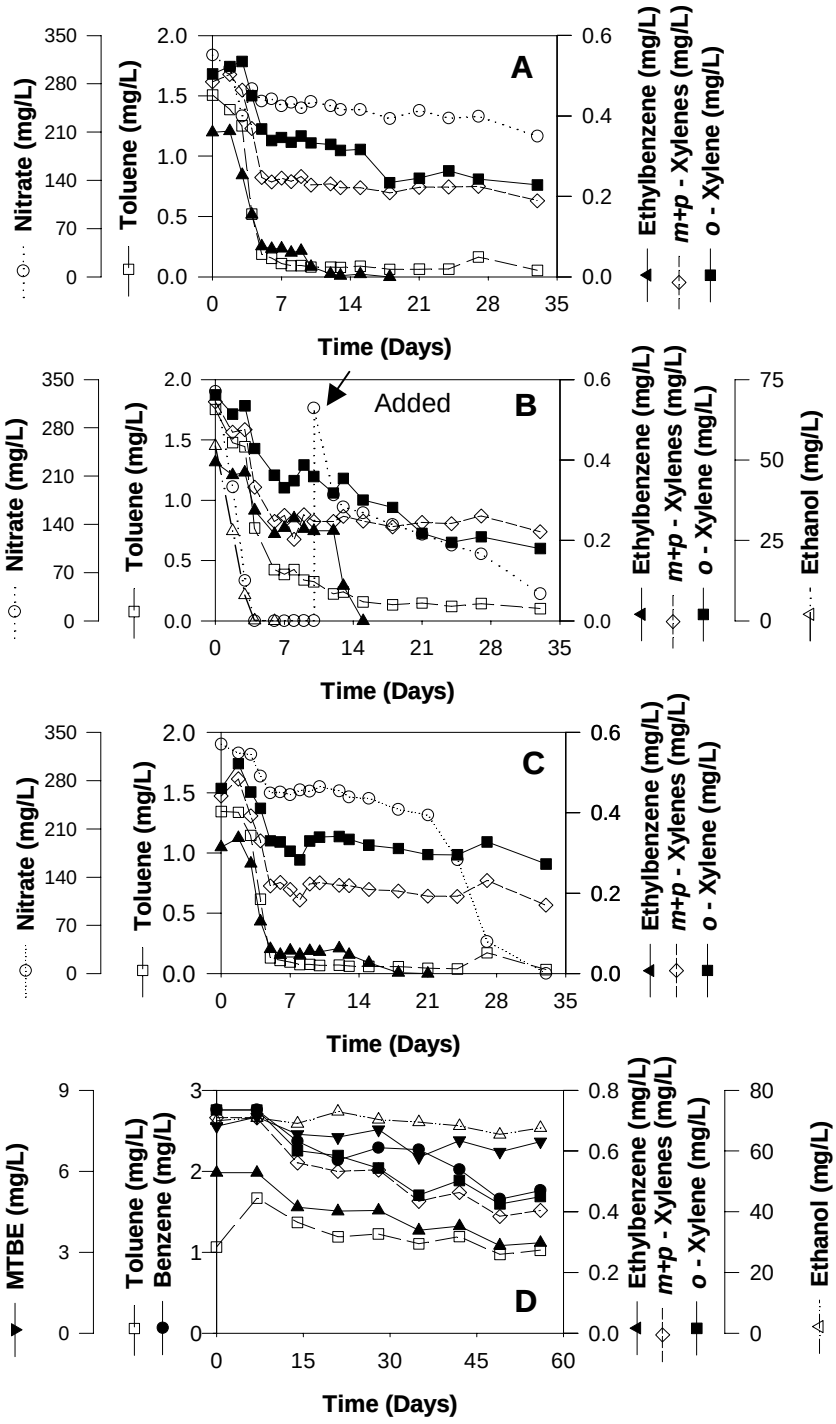


Figure 3-3. Degradation of BTEX and ethanol in denitrifying microcosms from Travis AFB amended with BTEX alone (A); BTEX plus ethanol (B); BTEX plus MtBE (C); and Control (D). Data points correspond to average values from three replicates.

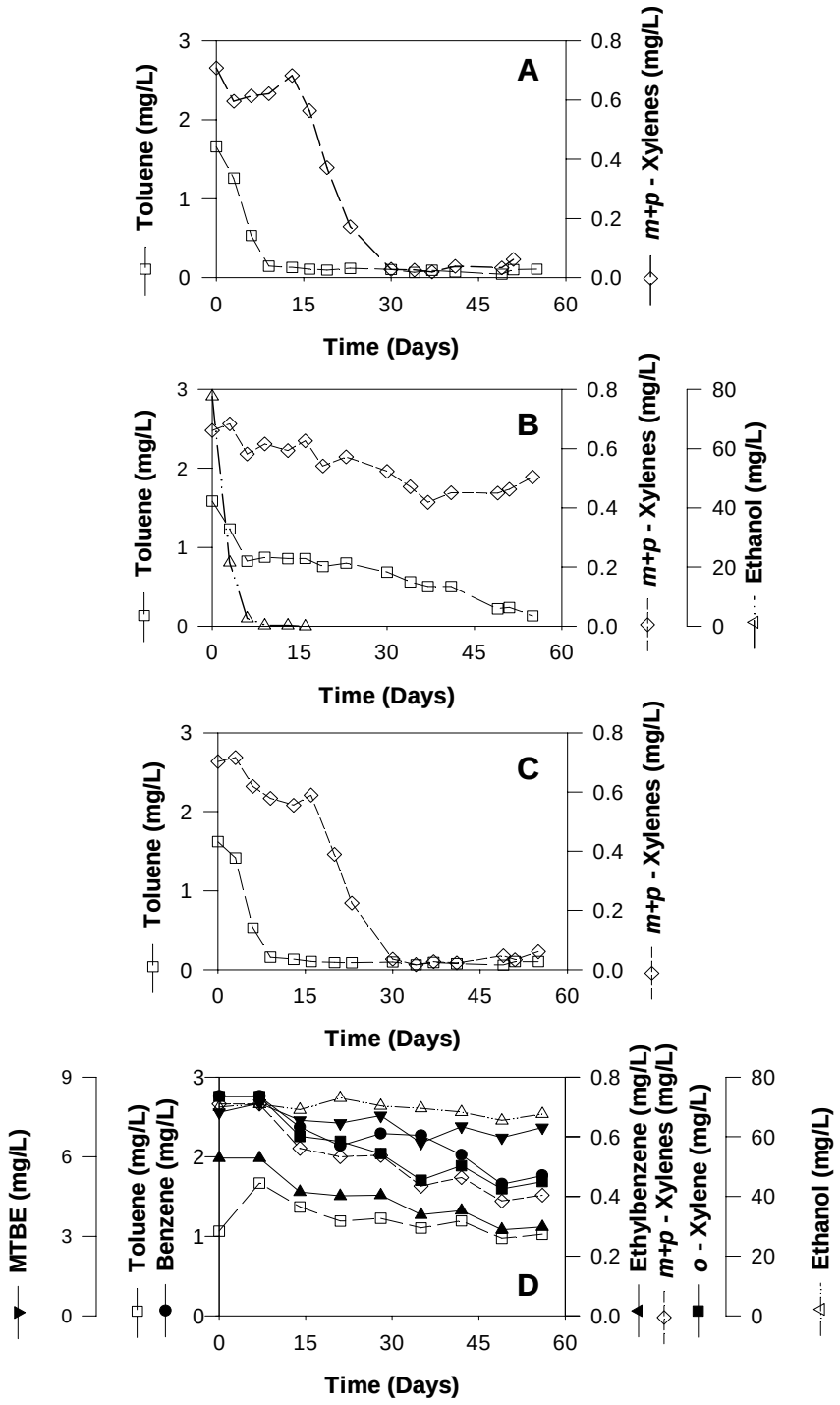


Figure 3-4. Degradation of BTEX and ethanol in iron (III)-amended microcosms from Travis AFB amended with BTEX alone (A); BTEX plus ethanol (B); BTEX plus MtBE (C); and Control (D). Data points correspond to average values from three replicates.

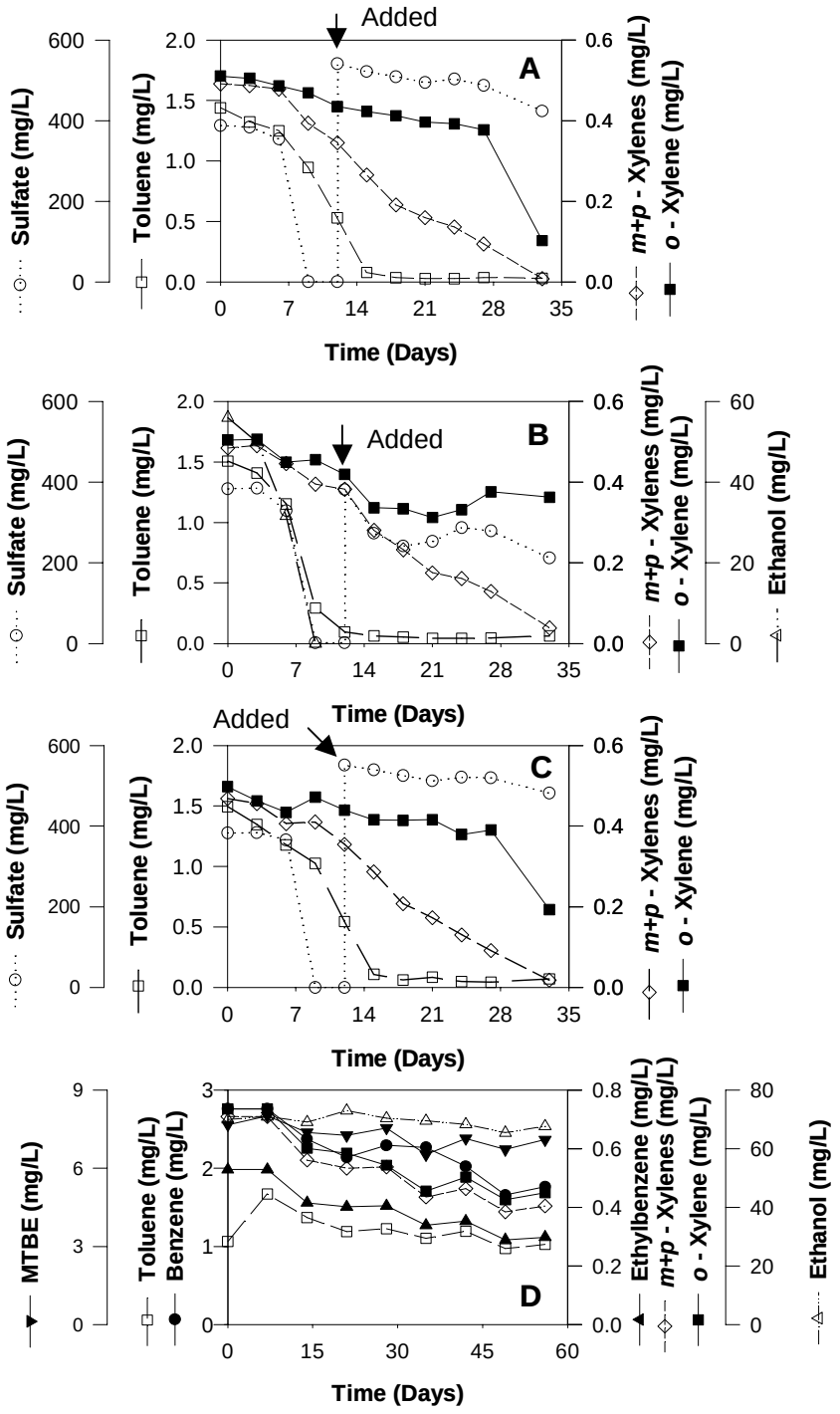


Figure 3-5. Degradation of BTEX and ethanol in sulfate-reducing microcosms from Travis AFB amended with BTEX alone (A); BTEX plus ethanol (B); BTEX plus MtBE (C); and Control (D). Data points correspond to average values from three replicates.

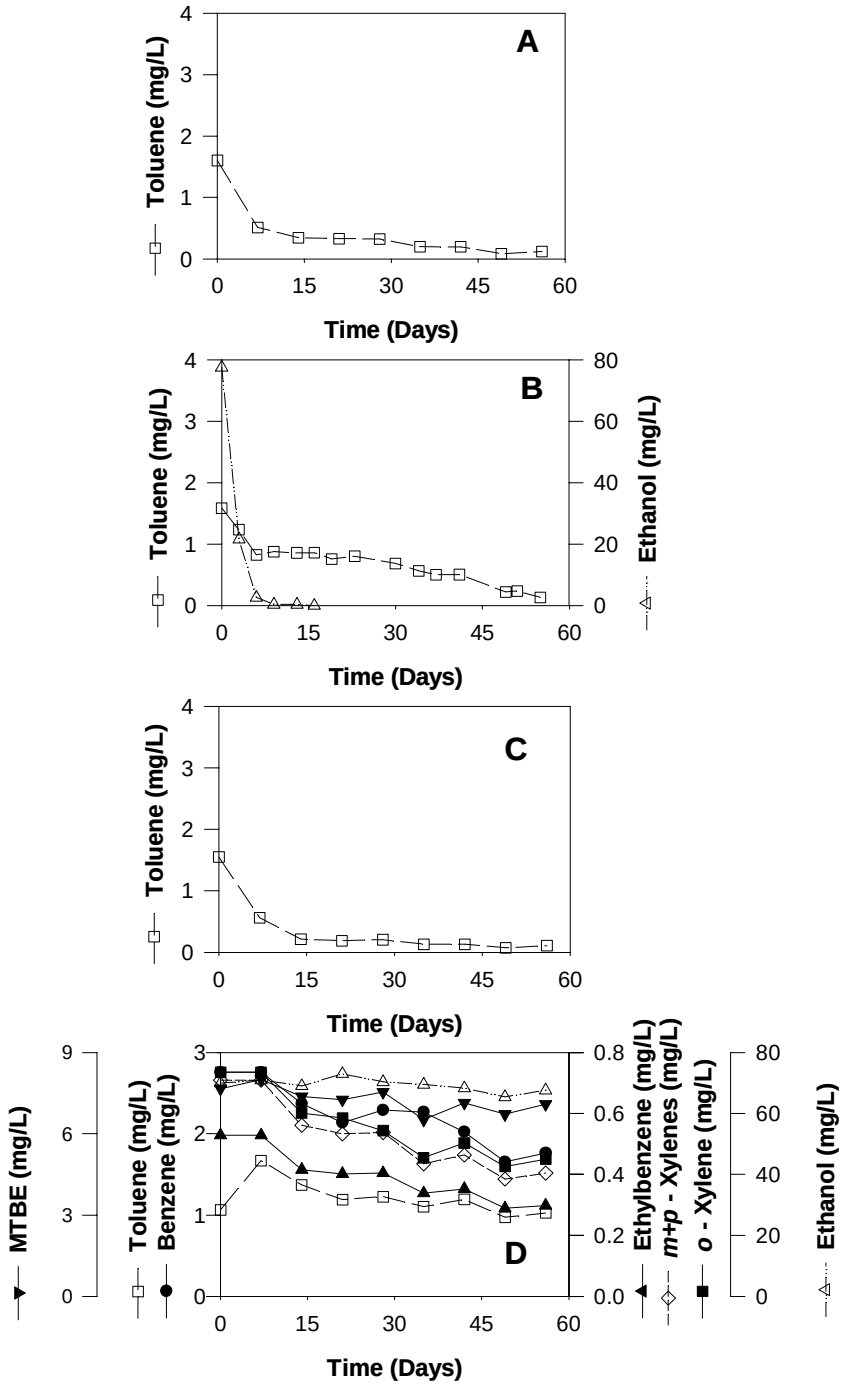


Figure 3-6. Degradation of toluene and ethanol in "methanogenic" microcosms from Travis AFB amended with BTEX alone (A); BTEX plus ethanol (B); BTEX plus MtBE (C); and Control (D). Data points correspond to average values from three replicates.

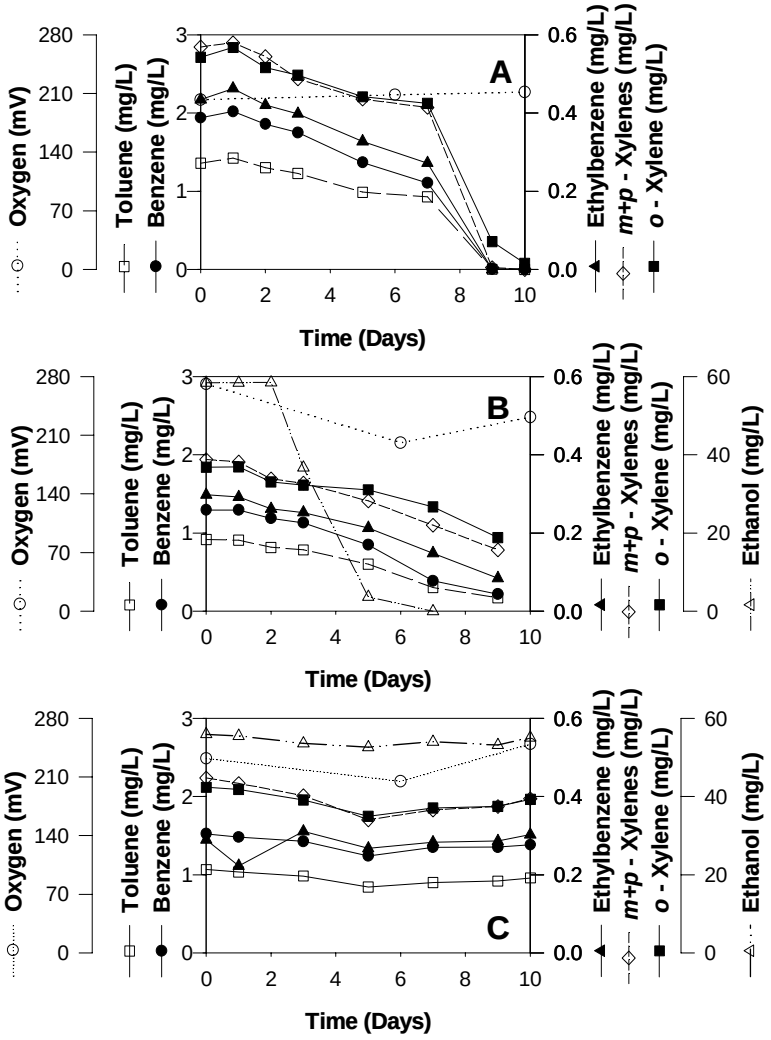


Figure 3-7. Degradation of BTEX and ethanol in aerobic microcosms from the Tracy site amended with BTEX alone (A); BTEX plus ethanol (B); and Control (C).

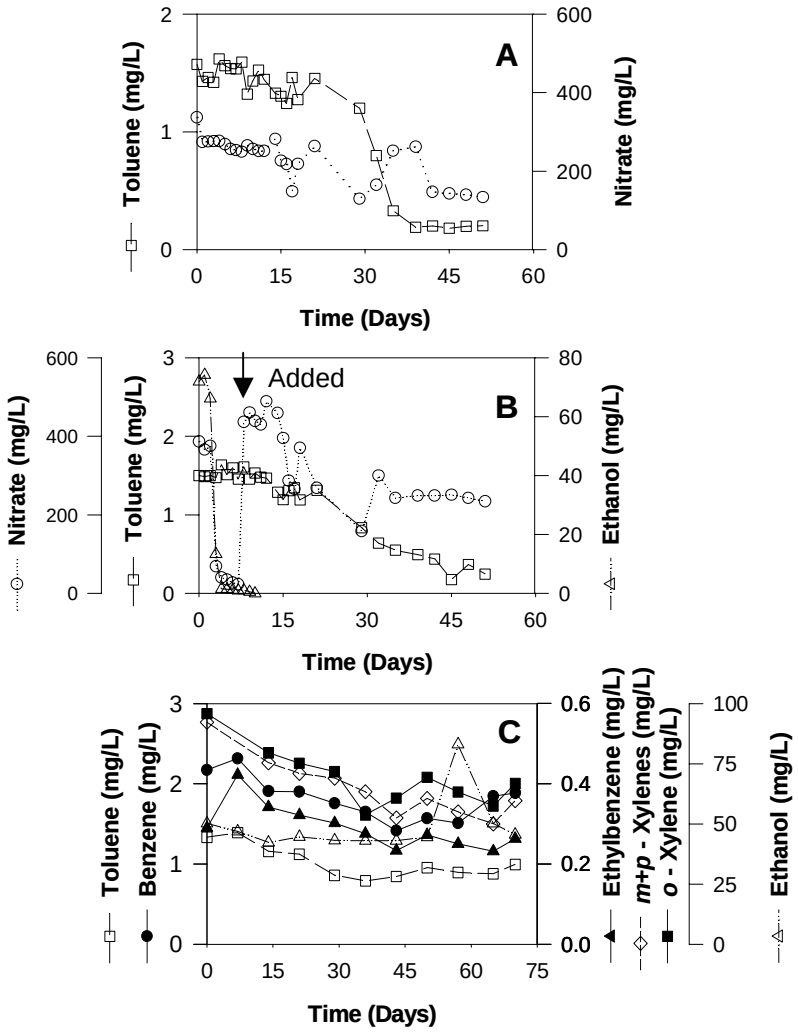


Figure 3-8. Degradation of toluene and ethanol in denitrifying microcosms from Tracy site amended with BTEX alone (A); BTEX plus ethanol (B); and Control (C). Data pots correspond to average values from three replicates**.**

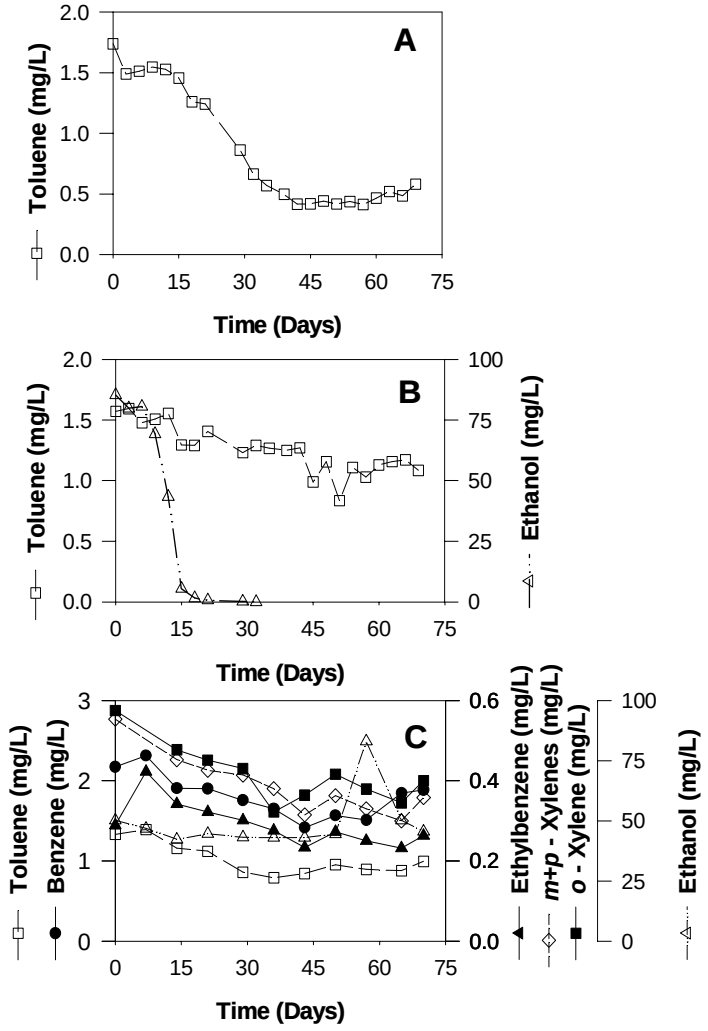


Figure 3-9. Degradation of toluene and ethanol in iron (III)-amended microcosms from Tracy site amended with BTEX alone (A); BTEX plus ethanol (B); and Control (C). Data points correspond to average values from three replicates.

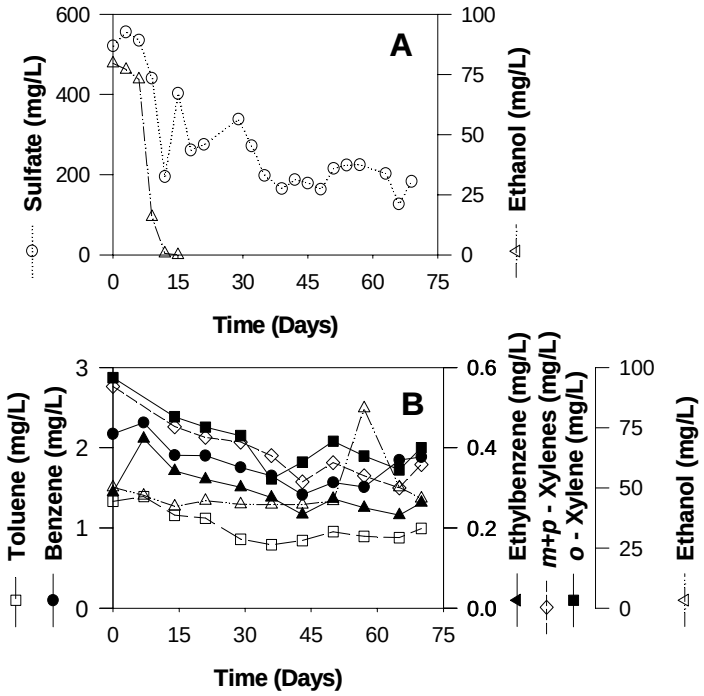


Figure 3-10. Degradation of ethanol in sulfate-reducing microcosms from the Tracy site amended with BTEX plus ethanol (A); and Control (B). Data points correspond to average values from three replicates.

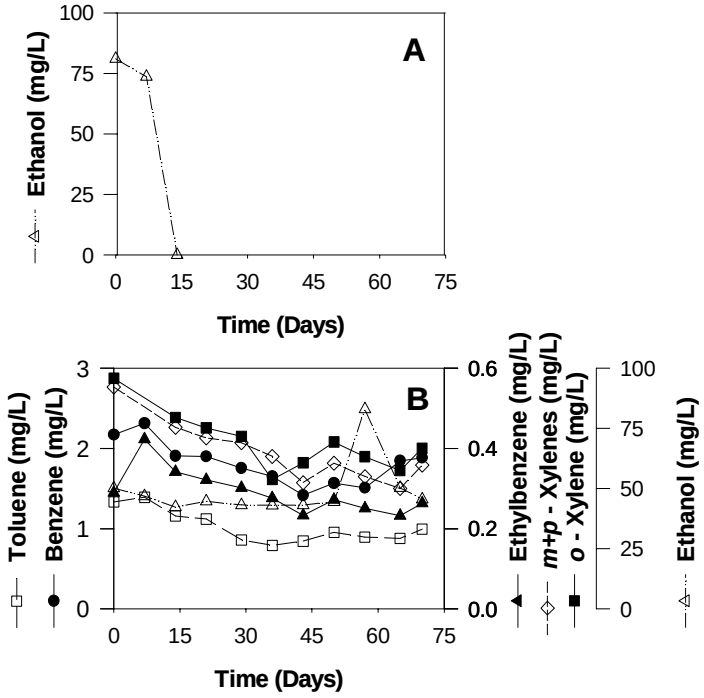


Figure 3-11. Degradation of ethanol in "methanogenic" microcosms from the Tracy site amended with BTEX plus ethanol (A); and Control (B). Data points correspond to average values from three replicates.

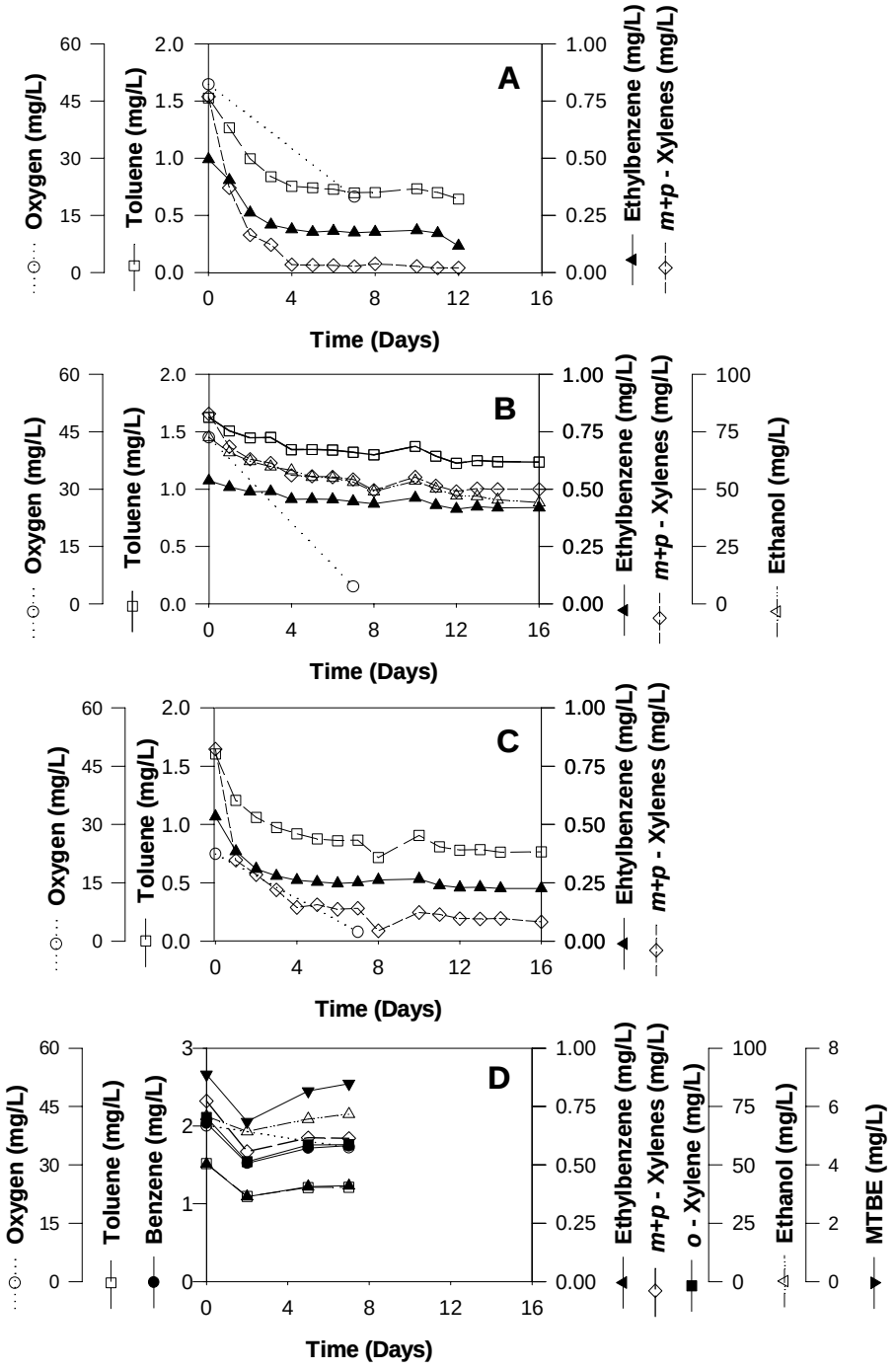


Figure 3-12. Degradation of BTEX and ethanol in aerobic microcosms from the Sacramento site amended with BTEX alone (A); BTEX plus ethanol (B); BTEX plus MtBE (C); and Control (D). Data points correspond to average values from three replicates.

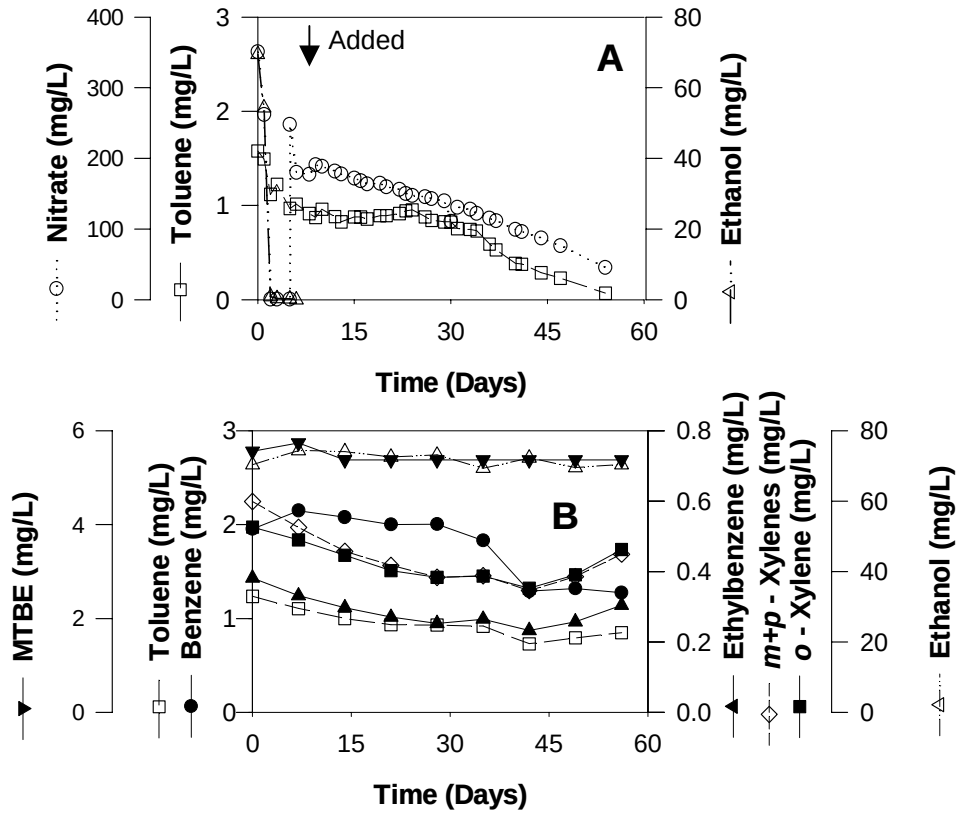


Figure 3-13. Degradation of toluene and ethanol in denitrifying microcosms from Sacramento site amended with BTEX plus ethanol (A); and Control (B). Data points correspond to average values from three replicates.

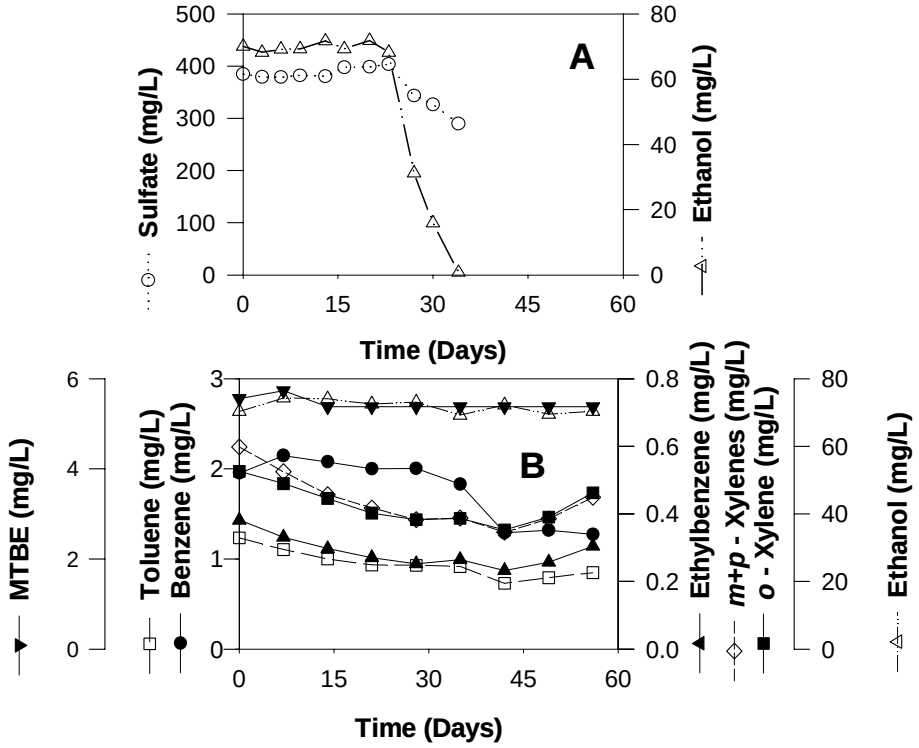


Figure 3-14. Degradation of ethanol in sulfate-reducing microcosms from the Sacramento site amended with BTEX plus ethanol (A); and Control (B). Data points correspond to average values from three replicates.

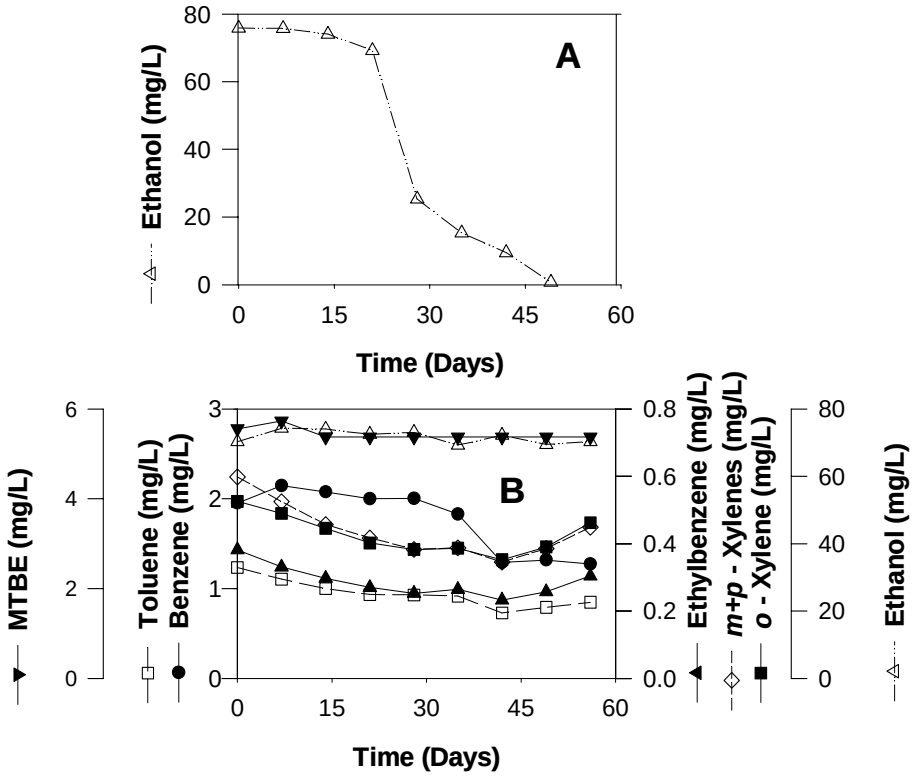


Figure 3-15. Degradation of ethanol in "methanogenic" microcosms from the Sacramento site amended with BTEX plus ethanol (A); and Control (B). Data points correspond to average values from three replicates.

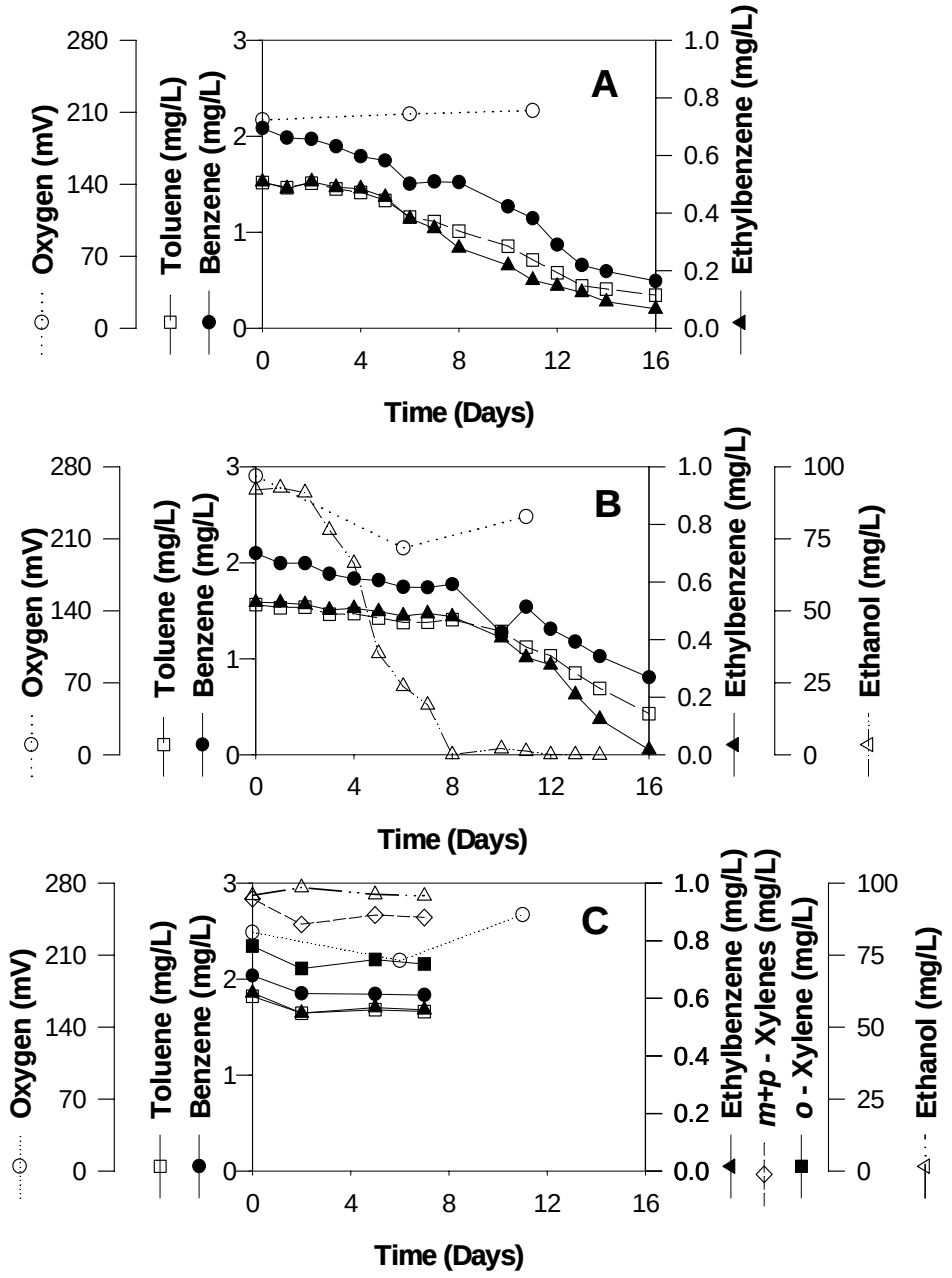


Figure 3-16. Degradation of BTEX and ethanol in aerobic microcosms from the Northwest Terminal site amended with BTEX alone (A); BTEX plus ethanol (B); and Control (C). Data points correspond to average values from three replicates.

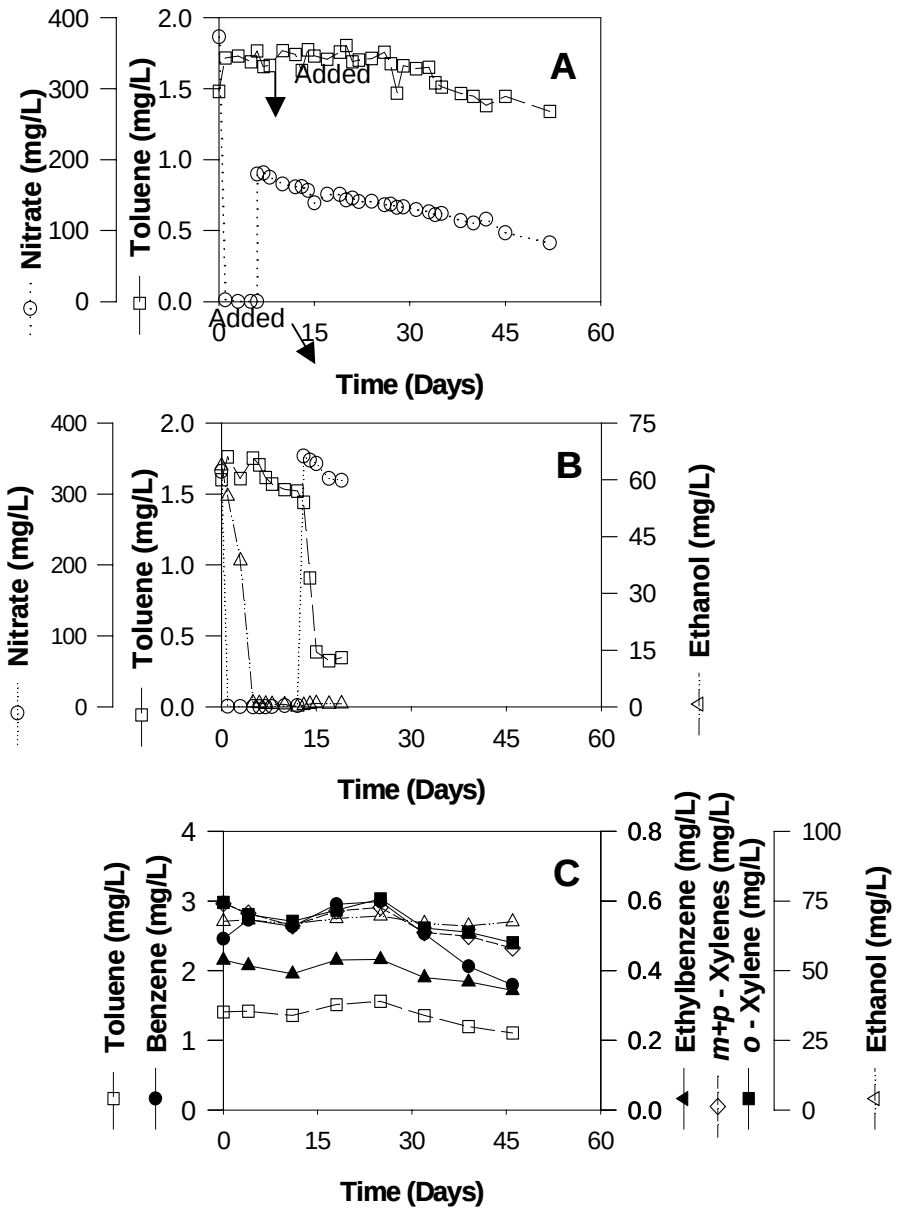


Figure 3-17. Degradation of toluene and ethanol in denitrifying microcosms from the Northwest Terminal site amended with BTEX alone (A); BTEX plus ethanol (B); and Control (C). Data points correspond to average values from three replicates.

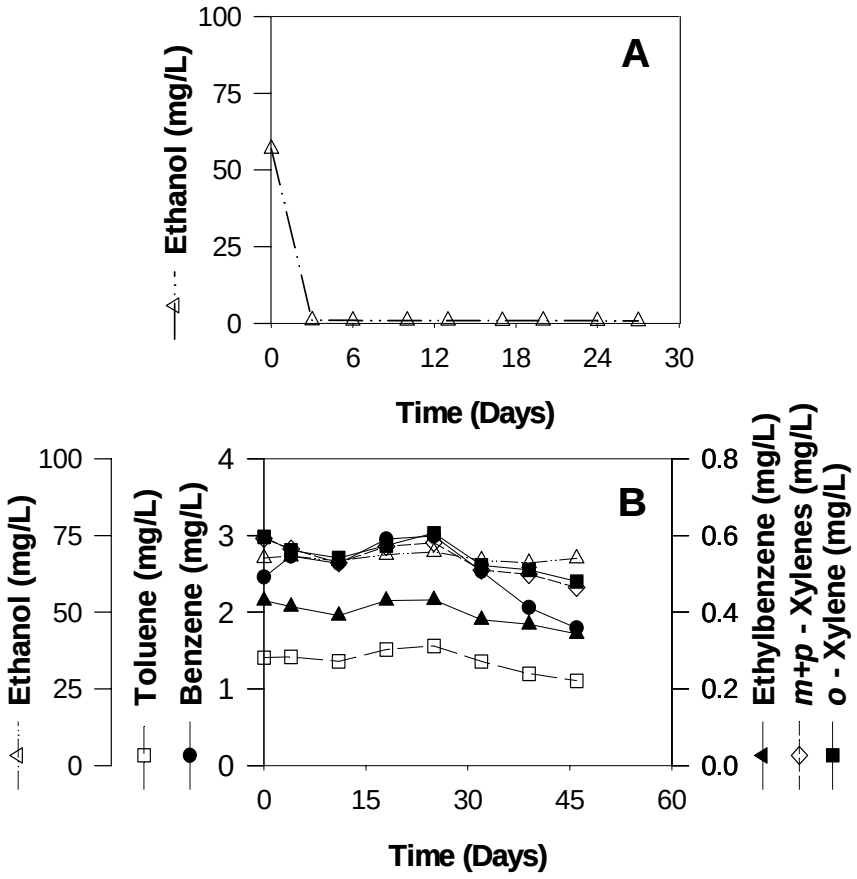


Figure 3-18. Degradation of ethanol in iron (III)-amended microcosms from the Northwest Terminal site amended with BTEX plus ethanol (A); and Control (B). Data points correspond to average values from three replicates.

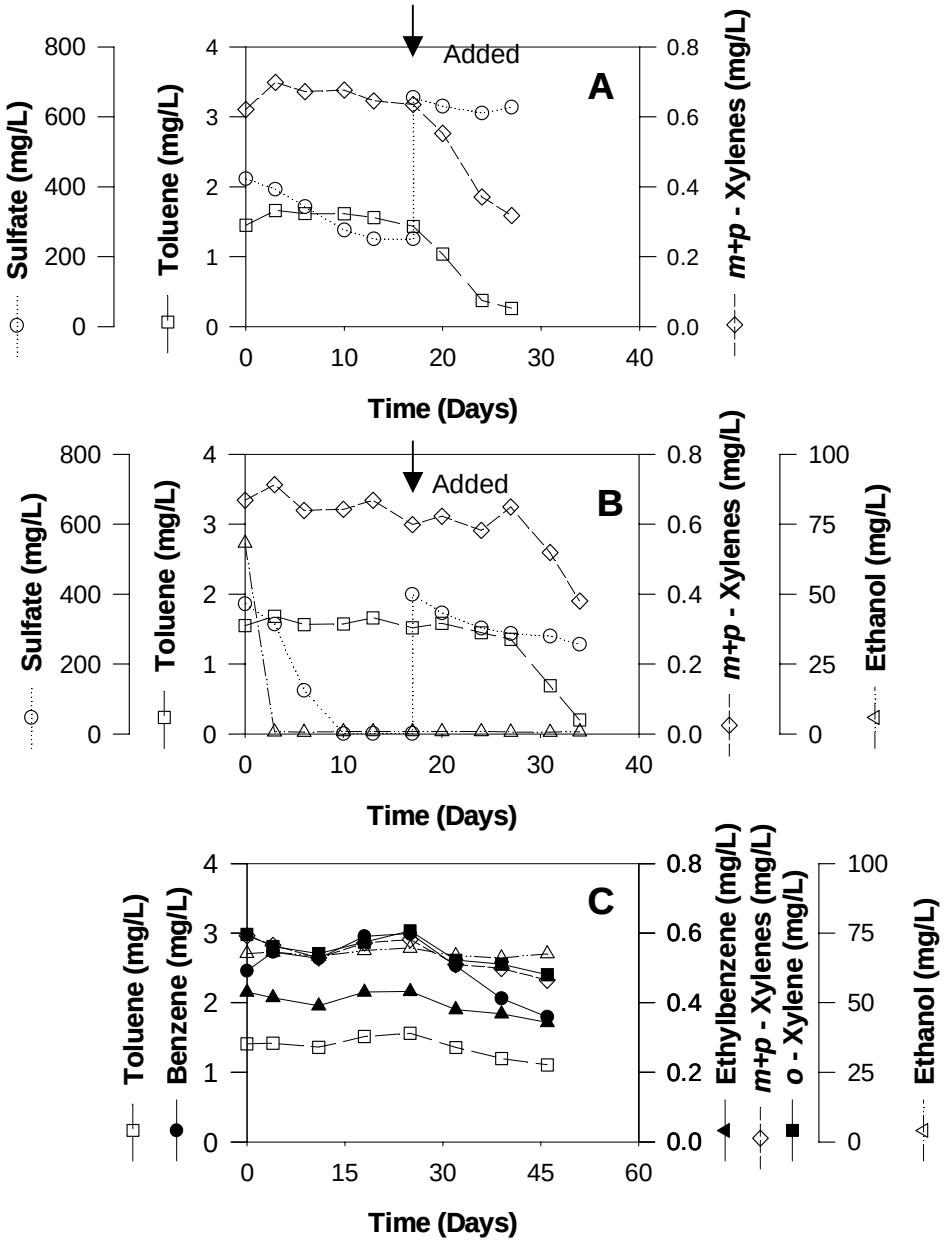


Figure 3-19. Degradation of toluene and ethanol in sulfate-reducing microcosms from the Northwest Terminal site amended with BTEX alone (A); BTEX plus ethanol (B); and Control (C). Data points correspond to average values from three replicates.

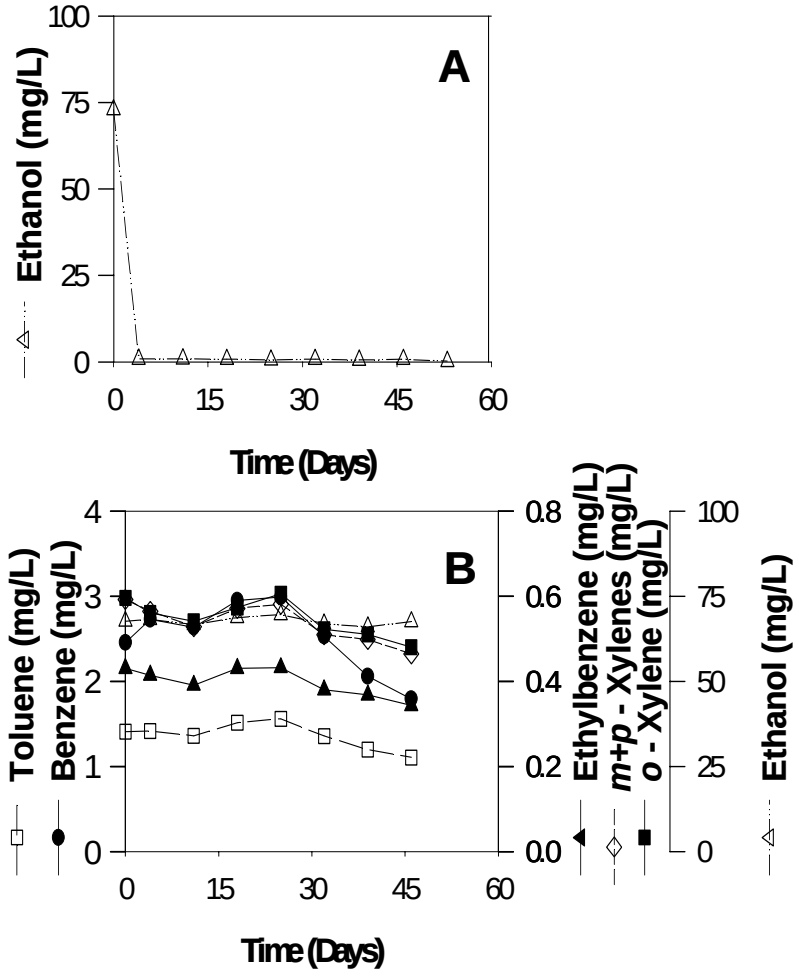


Figure 3-20. Degradation of ethanol in "methanogenic" microcosms from Northwest terminal site amended with BTEX plus ethanol (A); and Control (B). Data points correspond to average values from three replicates.

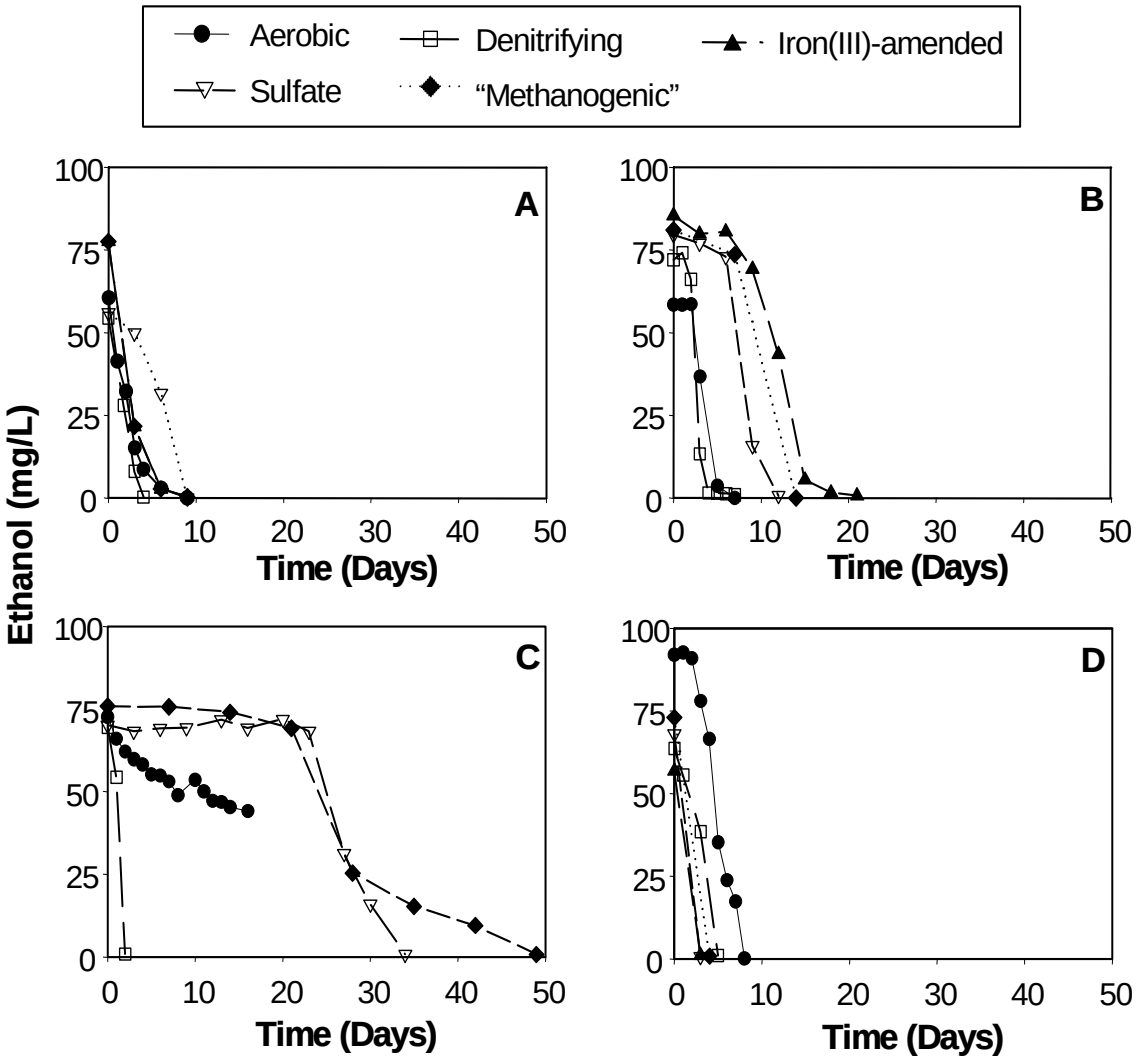


Figure 3-21. Ethanol degradation in microcosms from different sites under different electron-accepting conditions. Data points are averages from triplicate microcosms also amended with BTEX. A = Travis site; B = Tracy site; C = Sacramento site; D = Northwest Terminal site.

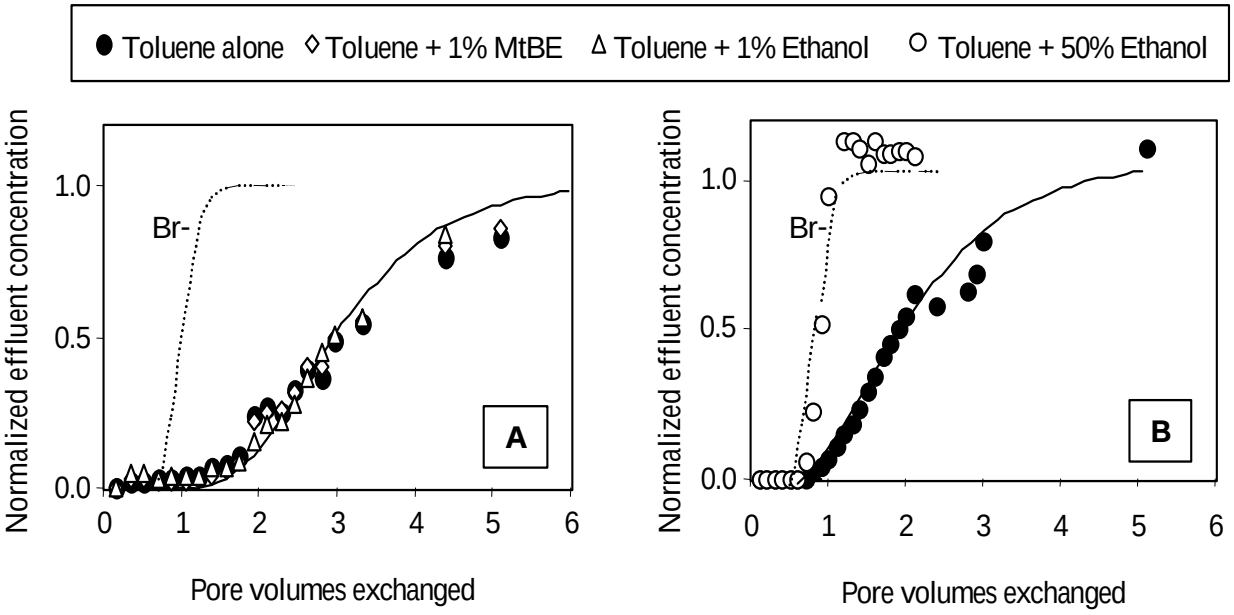


Figure 3-22. Retardation of toluene in columns amended with BTX alone and with 1% ethanol or MtBE (A) and with 50% ethanol (B). Solid lines represent the model fit Equation (3- 1).

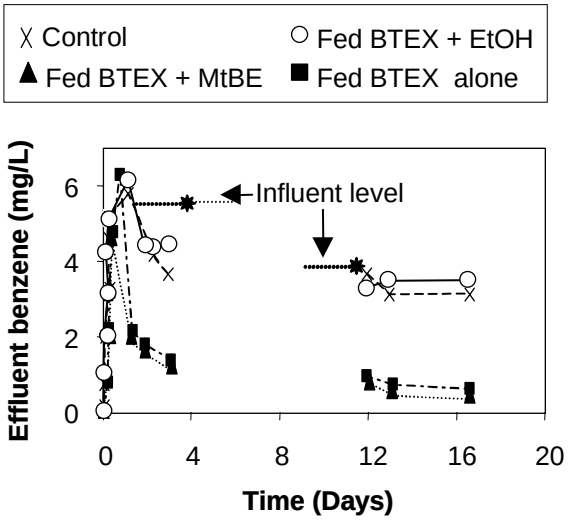


Figure 3-23. Benzene breakthrough from columns amended with BTEX alone, with ethanol (150 mg/L) or with MTBE (12 mg/L). The hydraulic retention time was 2.66 h.

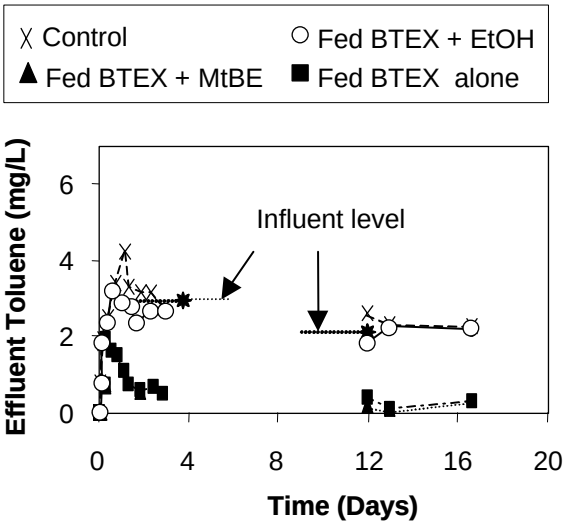


Figure 3-24. Toluene breakthrough from columns amended with BTEX alone, with ethanol (150 mg/L) or with MTBE (12 mg/L). The hydraulic retention time was 2.66 h.

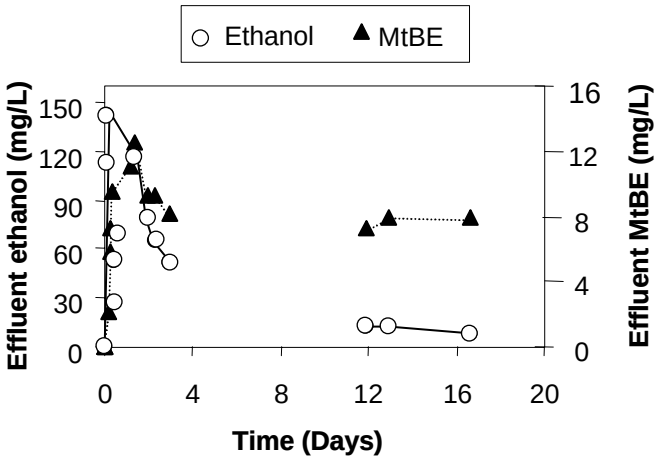


Figure 3-25. Ethanol and MtBE breakthrough from columns amended with BTEX and ethanol (150 mg/L) or with BTEX and MTBE (12 mg/L). The hydraulic retention time was 2.66 h.

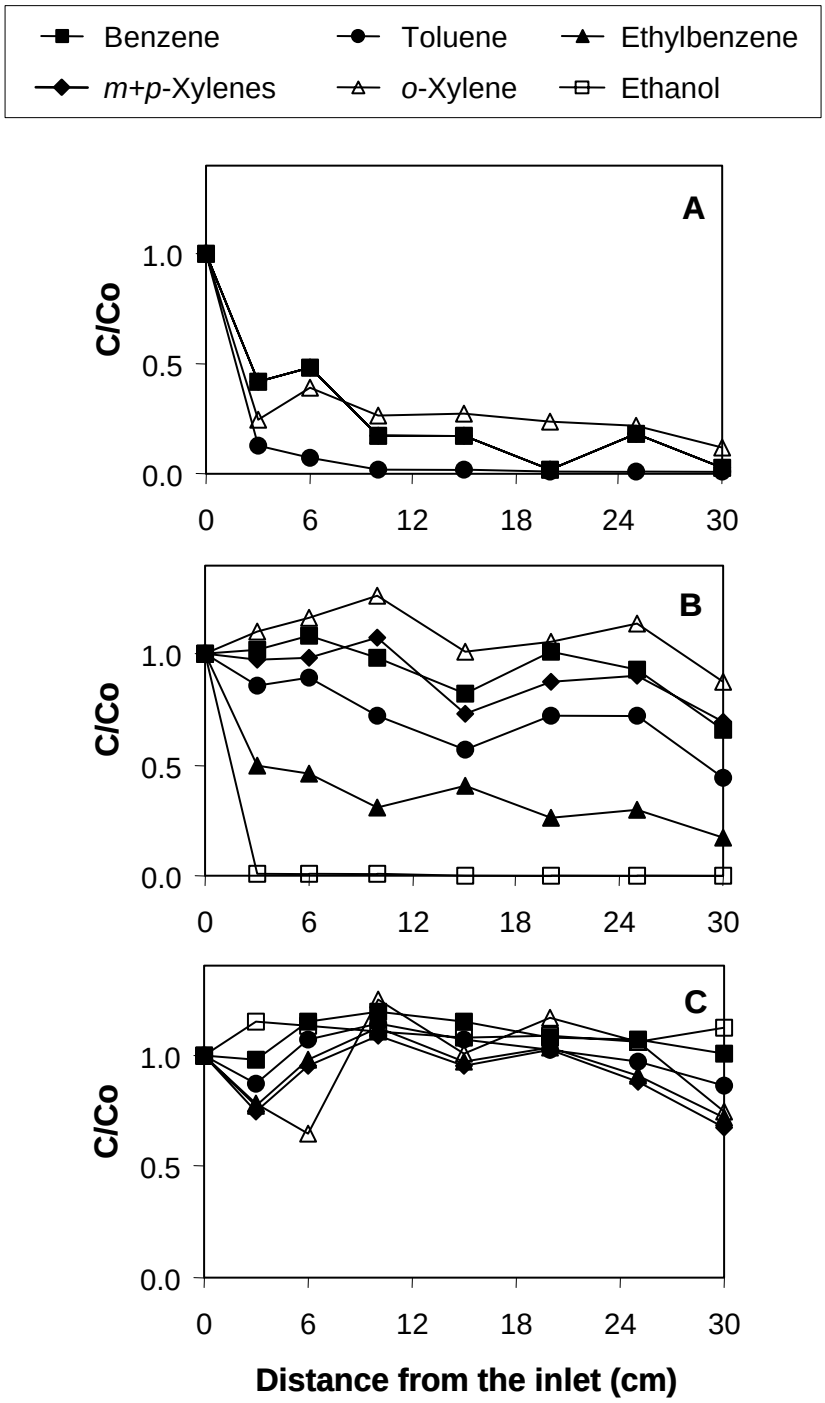


Figure 3-26. BTEX and ethanol concentration profiles in columns after 27 days of operation. Panel A corresponds to columns amended with BTEX alone (benzene 5.2 mg/L, toluene 4.1 mg/L, ethylbenzene 2.3 mg/L, m+p-xylenes 2.4 mg/L, o-xylene 2.5 mg/L); Panel B is BTEX plus ethanol (100 mg/L); and Panel C is the poisoned control.

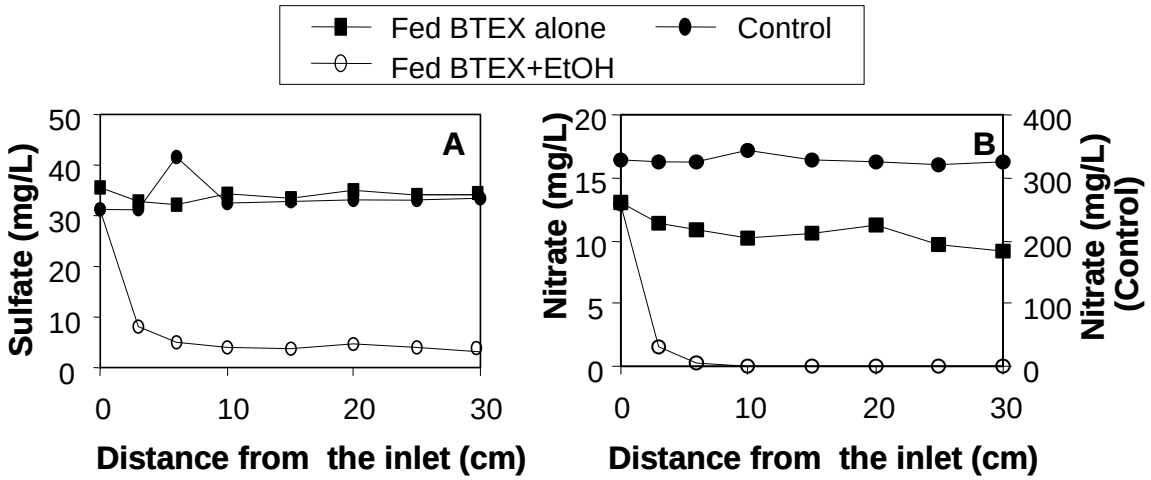


Figure 3-27. Sulfate (A) and nitrate (B) concentration profiles in columns amended with BTEX alone or with ethanol after 27 days. A higher nitrate level in the control was contributed by the Kathon biocide.

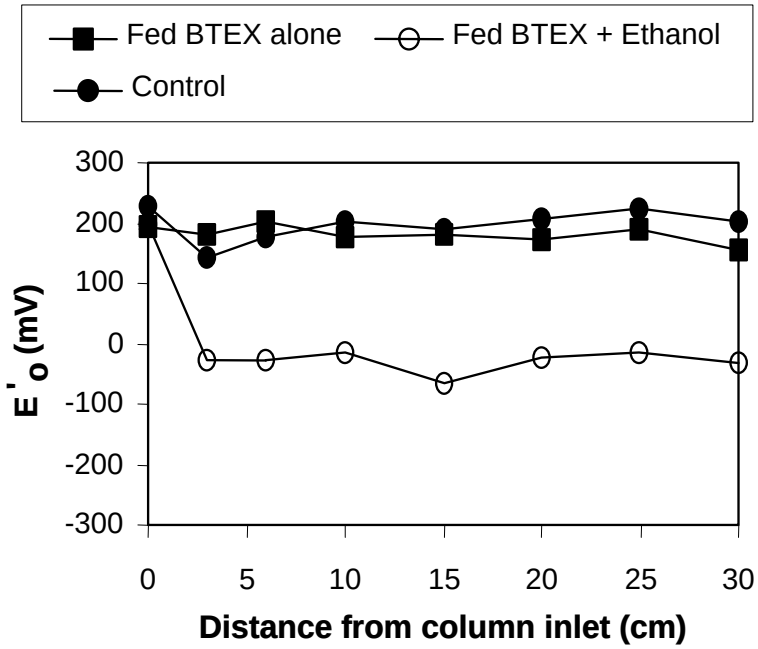


Figure 3-28. Oxidation-reduction potential profiles after 27 days of operation.

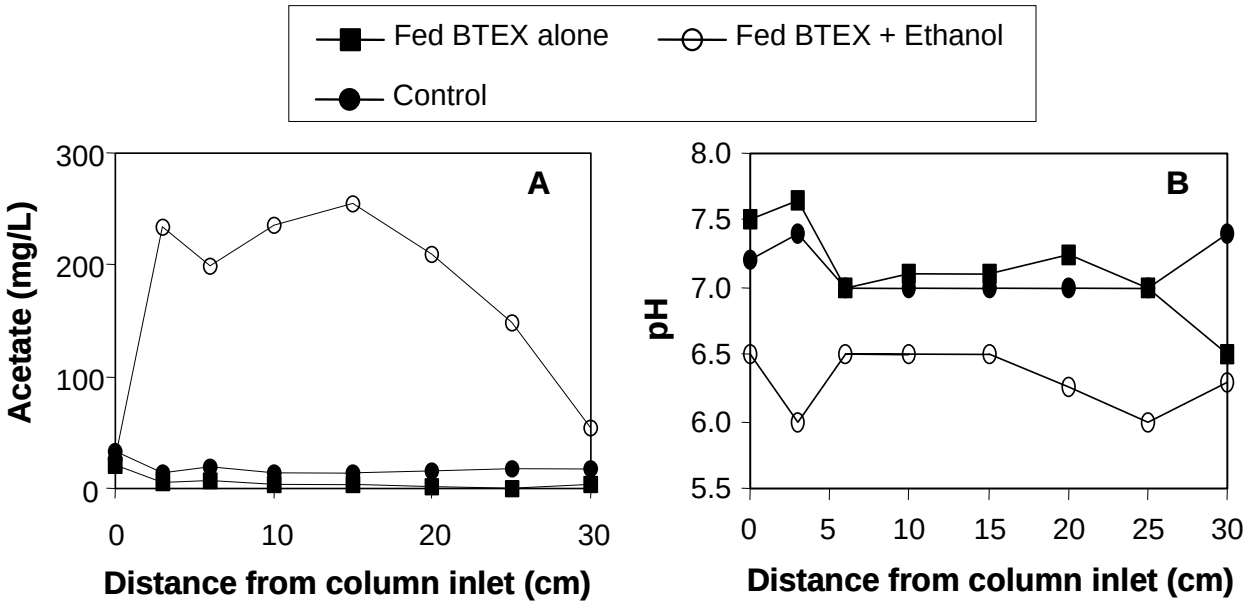


Figure 3-29. Acetate production (A) and pH (B) profiles in columns amended with BTEX alone; BTEX plus ethanol; and control; after 27 days of operation.

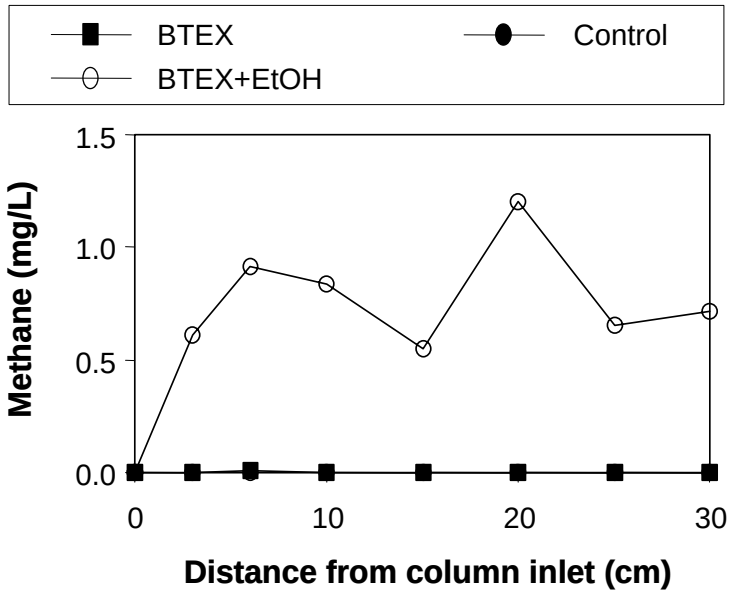
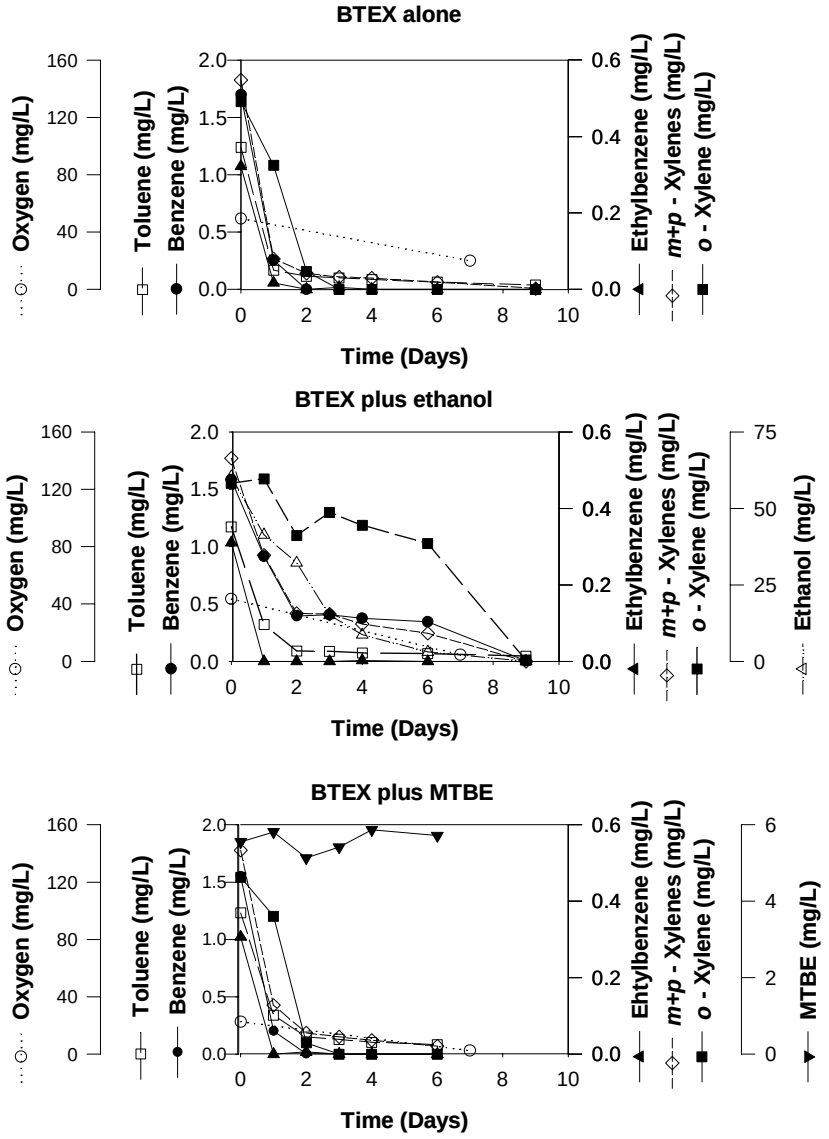


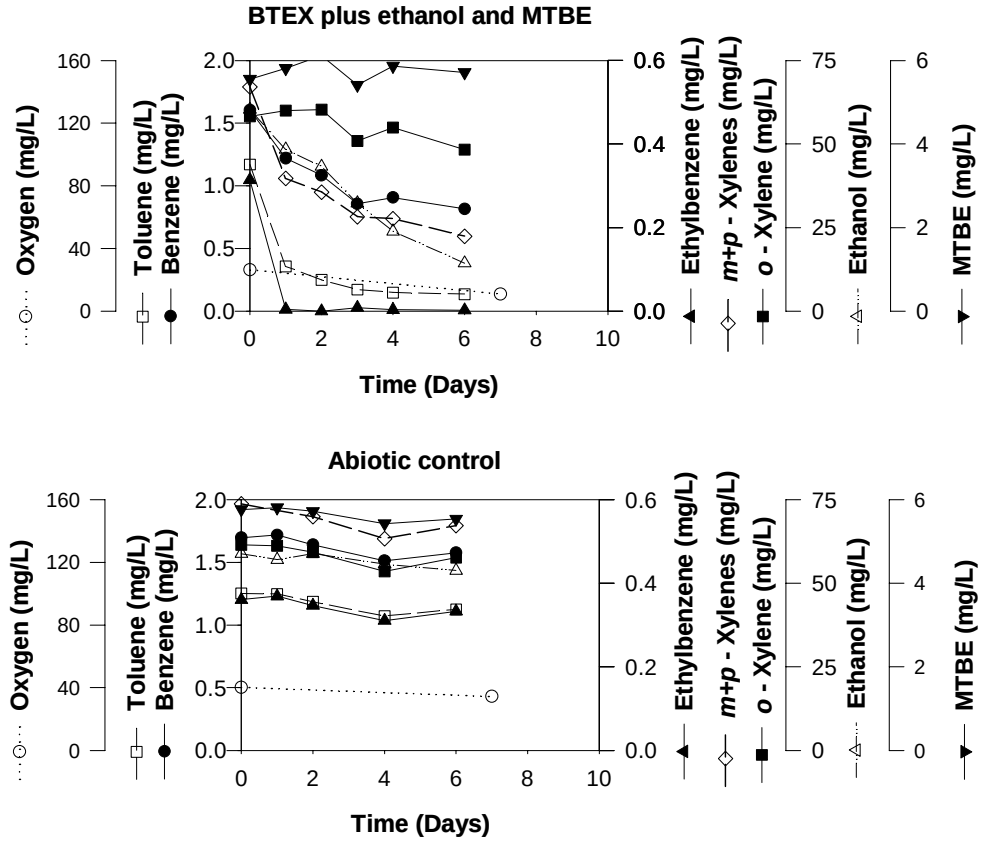
Figure 3-30. Methane profiles along the columns after 27 days of operation.

Appendix A
Travis AFB Microcosm Data

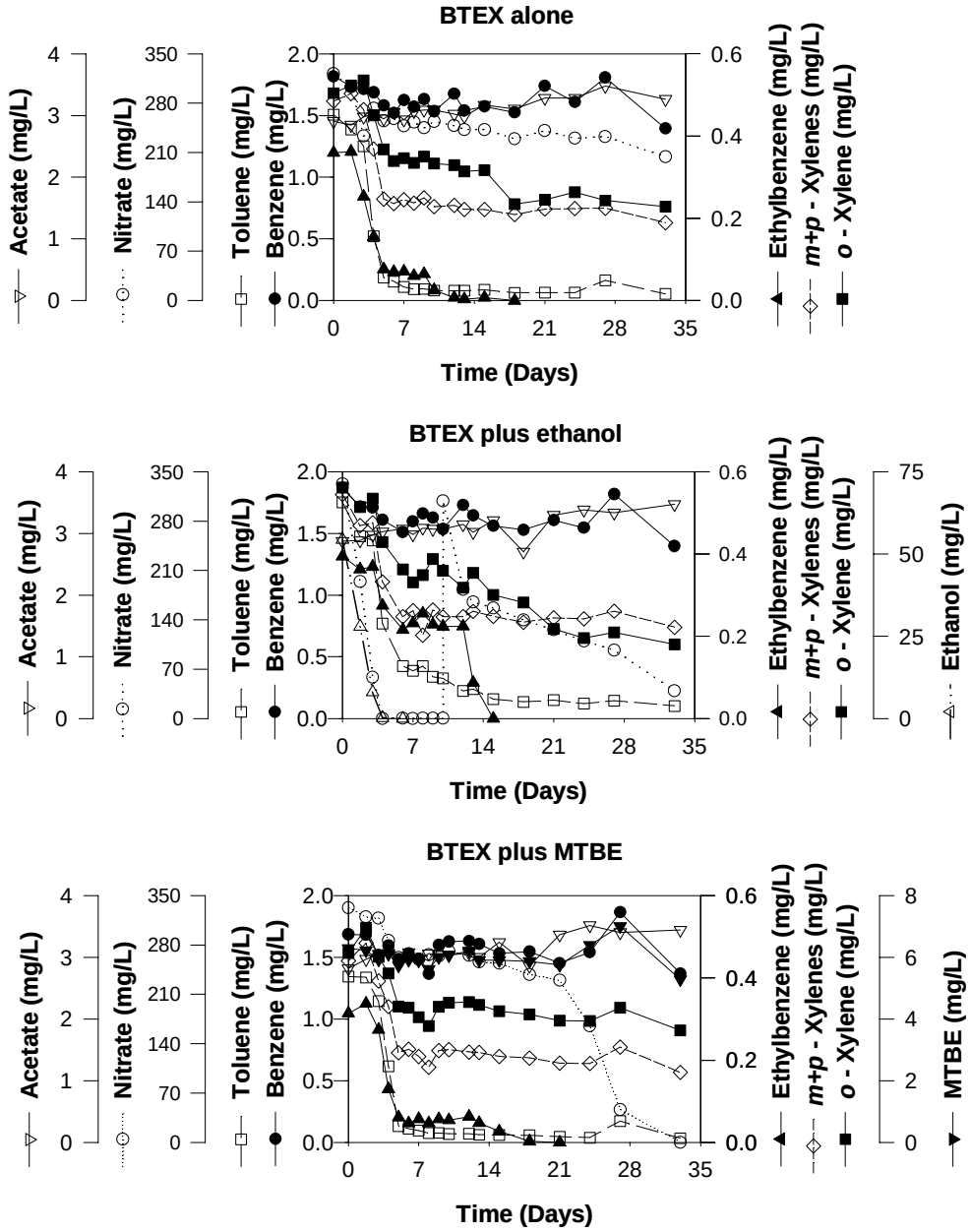
TRAVIS AFB SITE, *Aerobic Conditions*



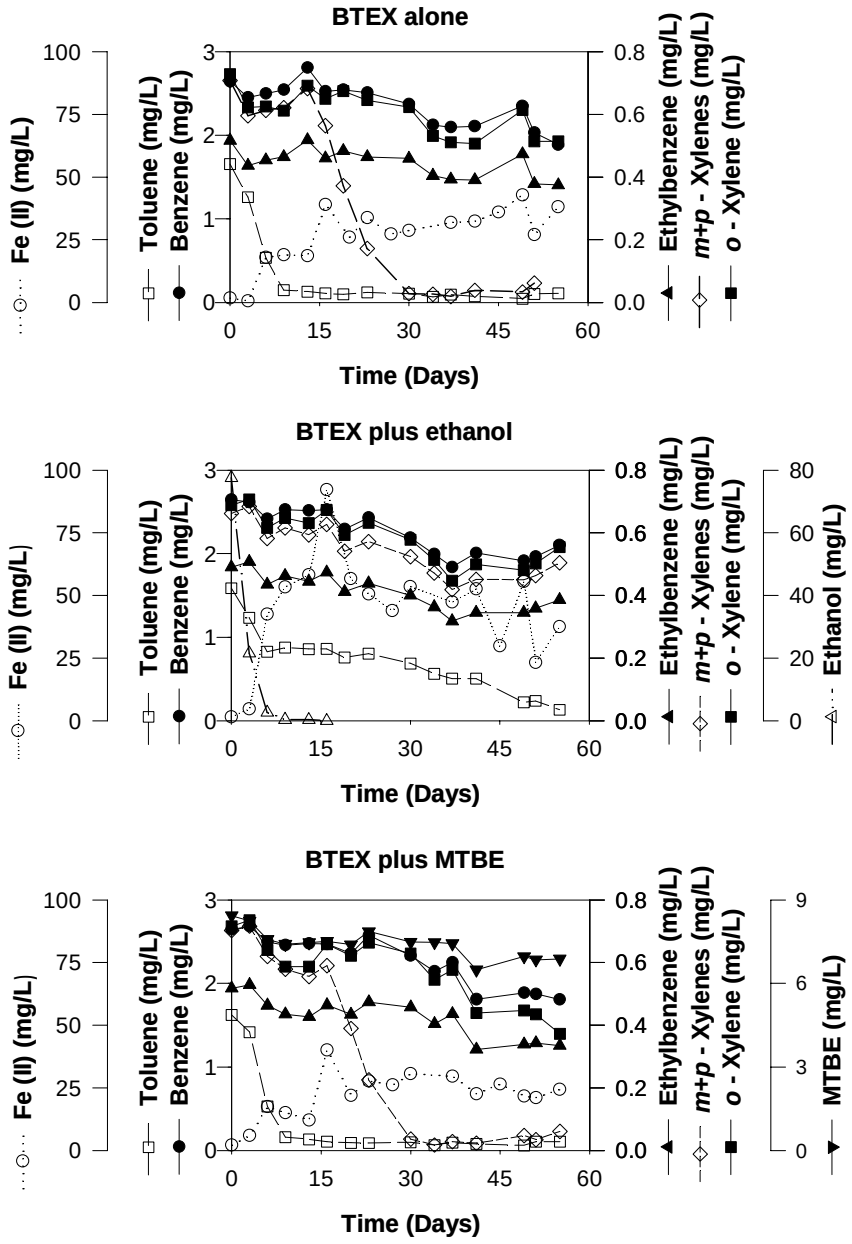
TRAVIS AFB SITE, *Aerobic Conditions*



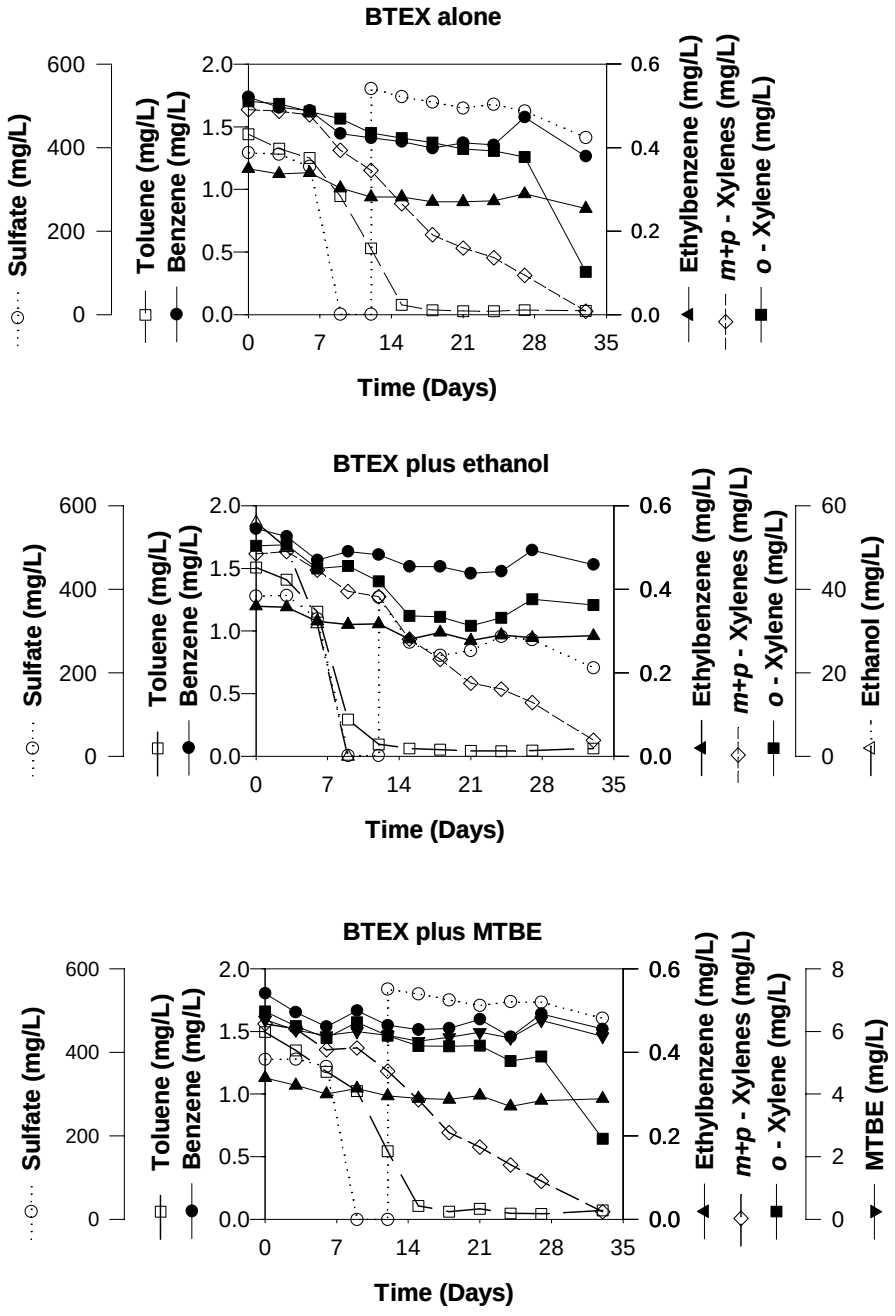
TRAVIS AFB SITE, *Denitrifying Conditions*



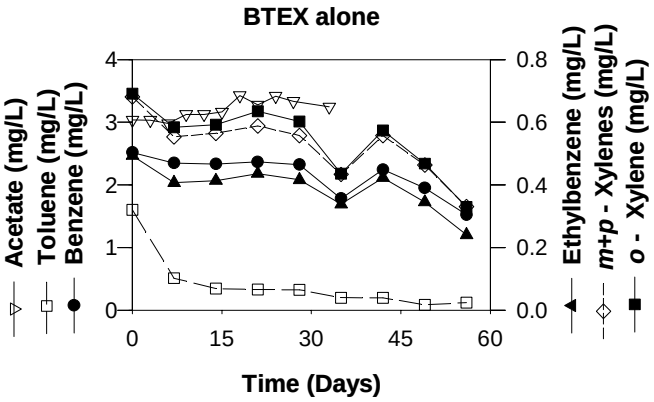
TRAVIS AFB SITE, *Iron (III)-amended Conditions*

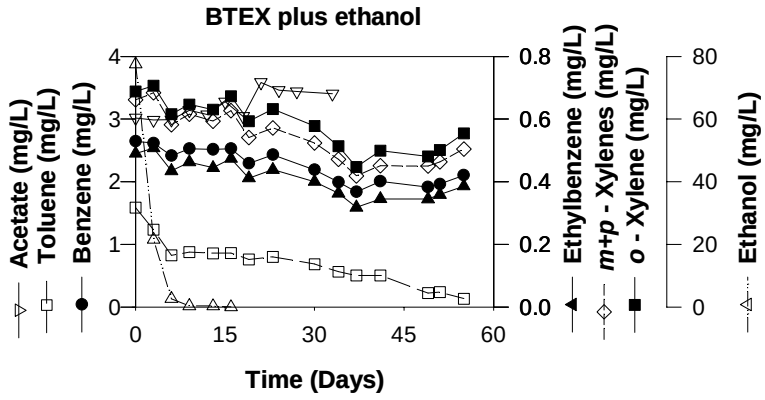


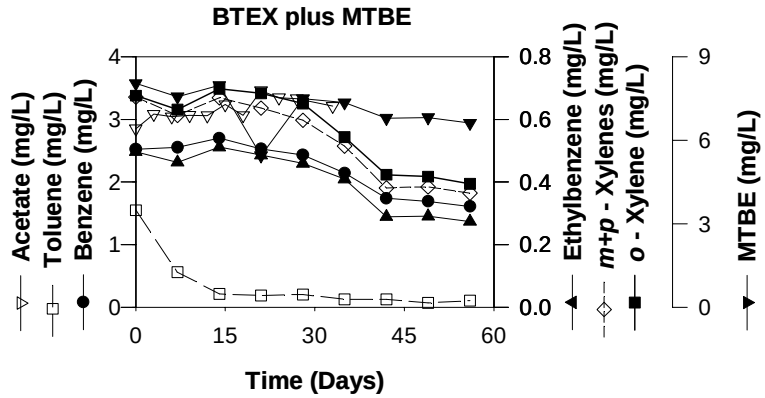
TRAVIS AFB SITE, *Sulfate-reducing conditions*



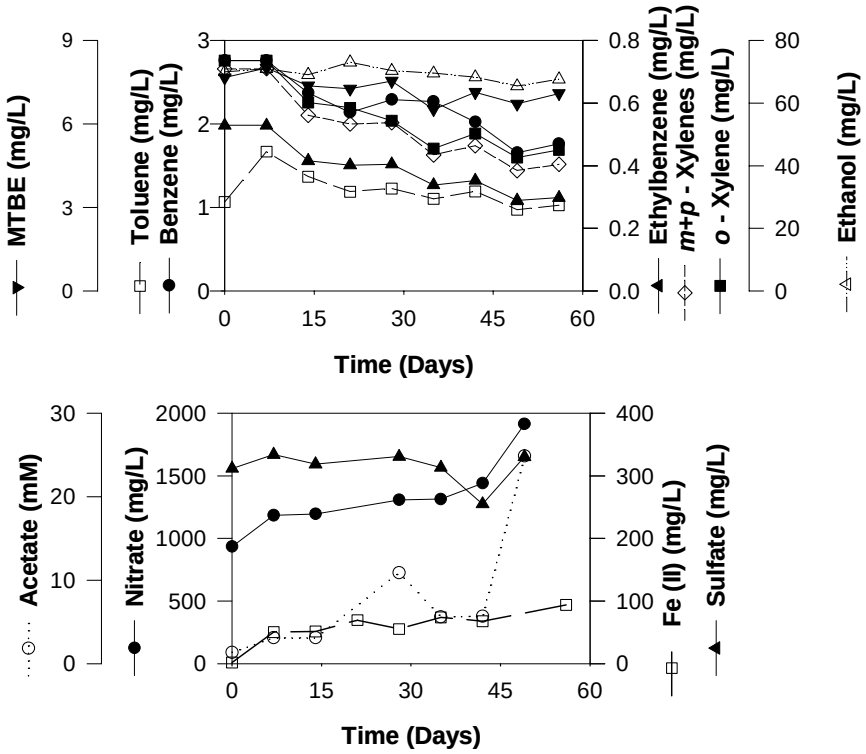
TRAVIS AFB SITE, *"Methanogenic" Conditions*







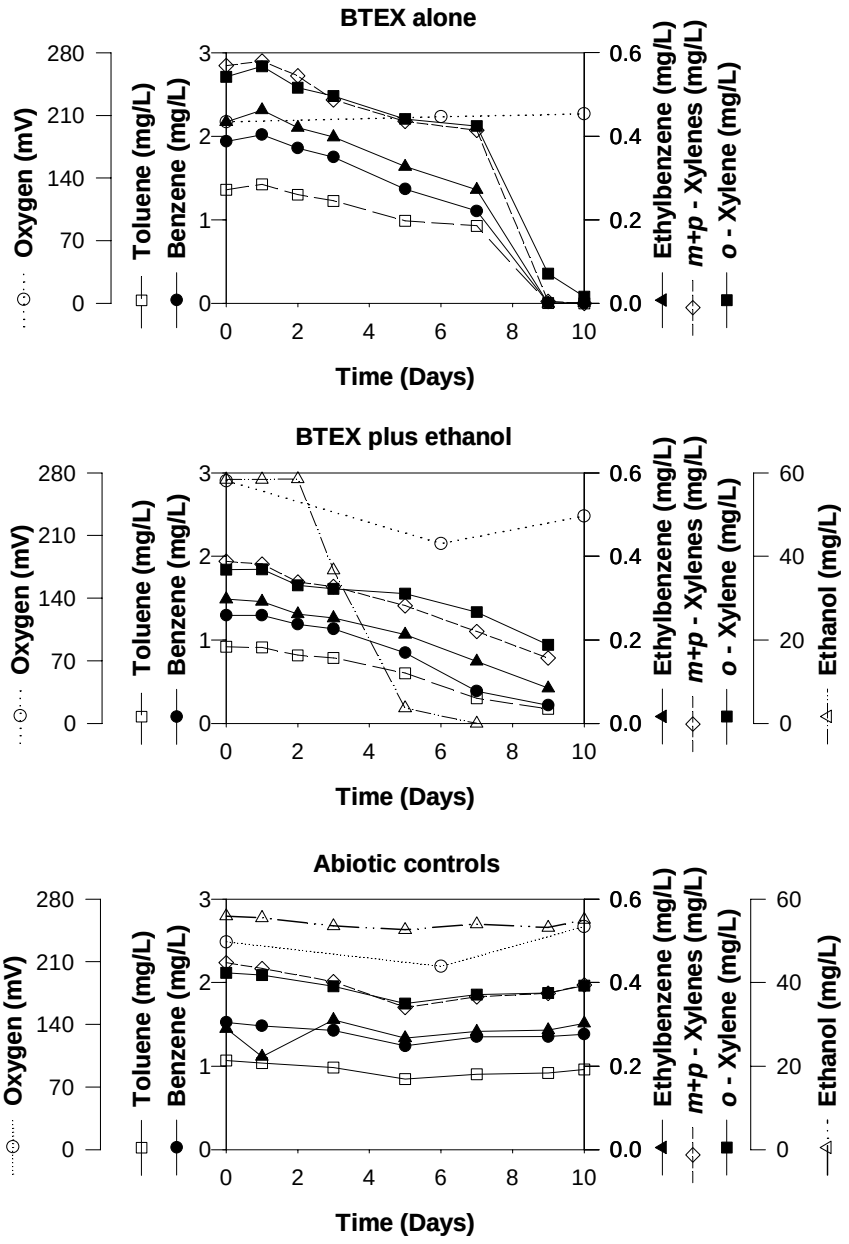
TRAVIS AFB SITE, *Abiotic Controls*



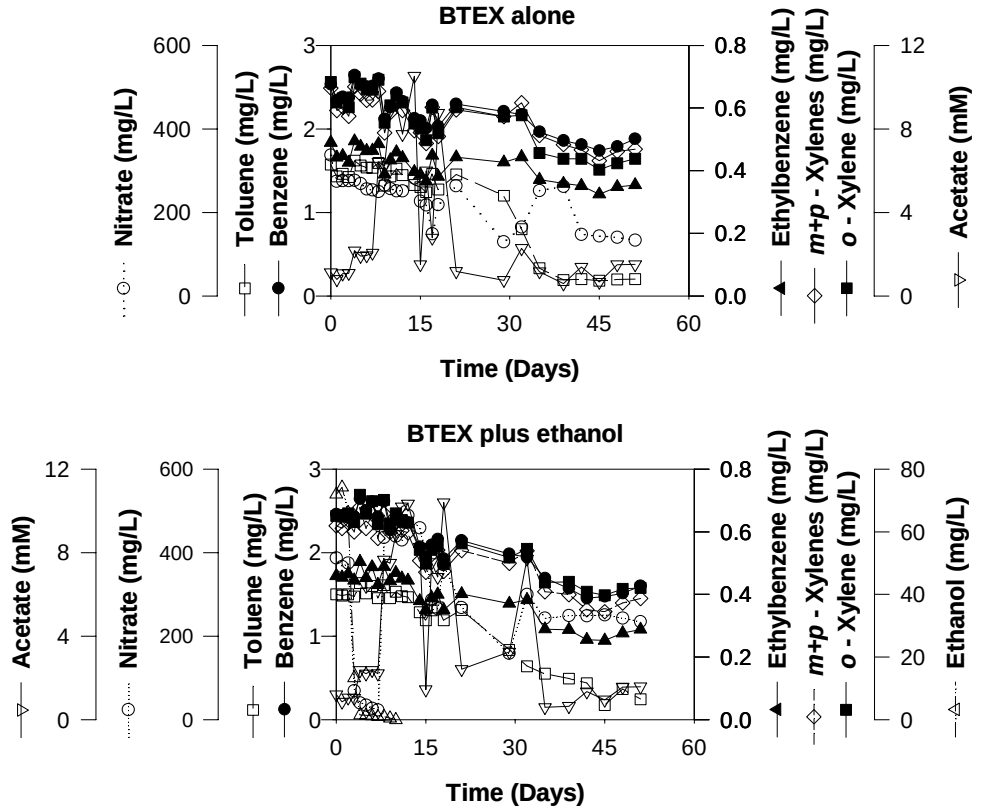
Note that it was difficult to maintain the controls sterile, as suggested by decreasing BTEX concentrations. Difficulties to keep soil sterile are commonly reported in the literature (e.g., Fava et al., 1998), where heat, $HgCl_2$ and NaN_3 have tested without complete success. Repeated poisoning with a the Kathon biocide partially alleviated this problem. The nitrate concentration increased during incubation period since Kathon contains magnesium nitrate.

Appendix B
Tracy Site Microcosm Data

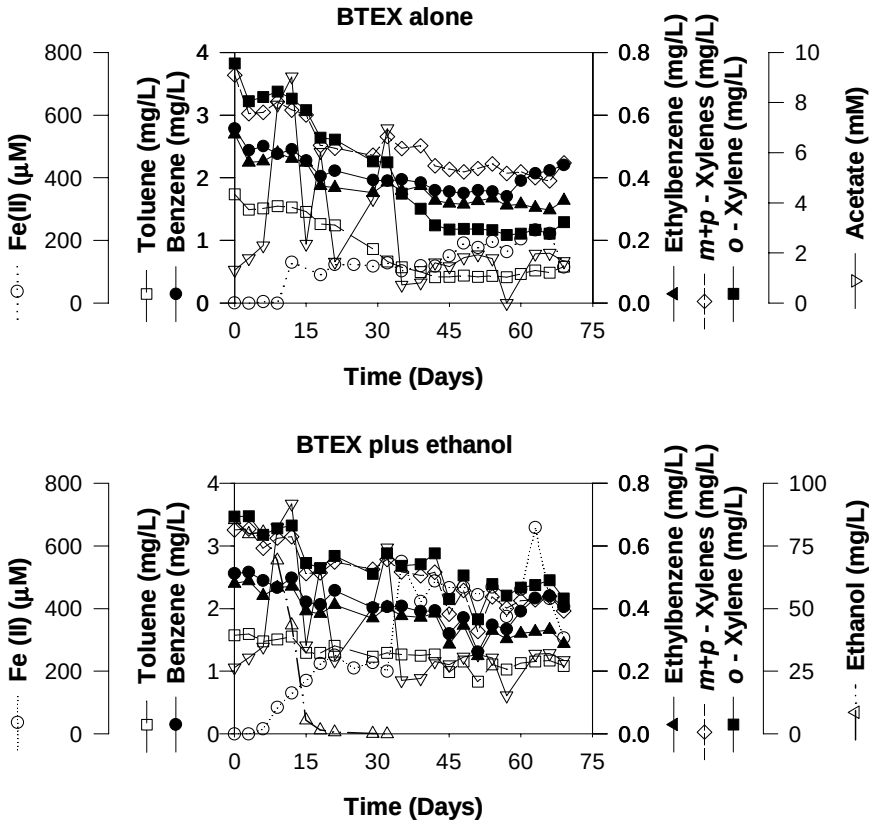
TRAYC SITE, *Aerobic Conditions*



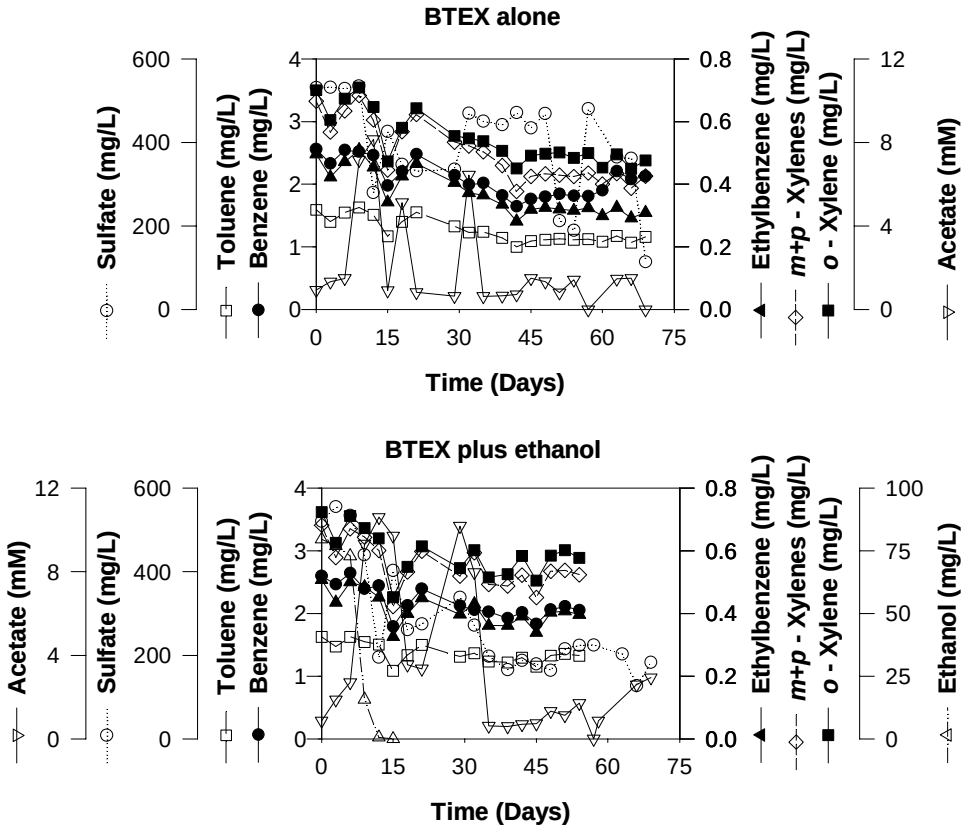
TRACY SITE, *Denitrifying Conditions*



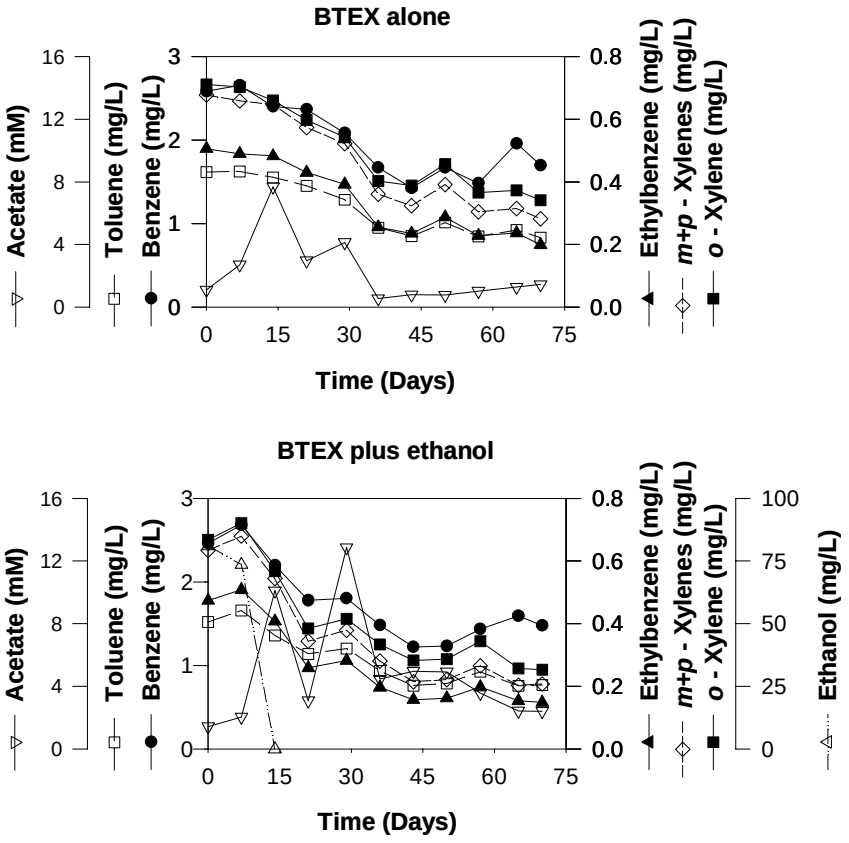
TRACY SITE, *Iron (III)-amended Conditions*

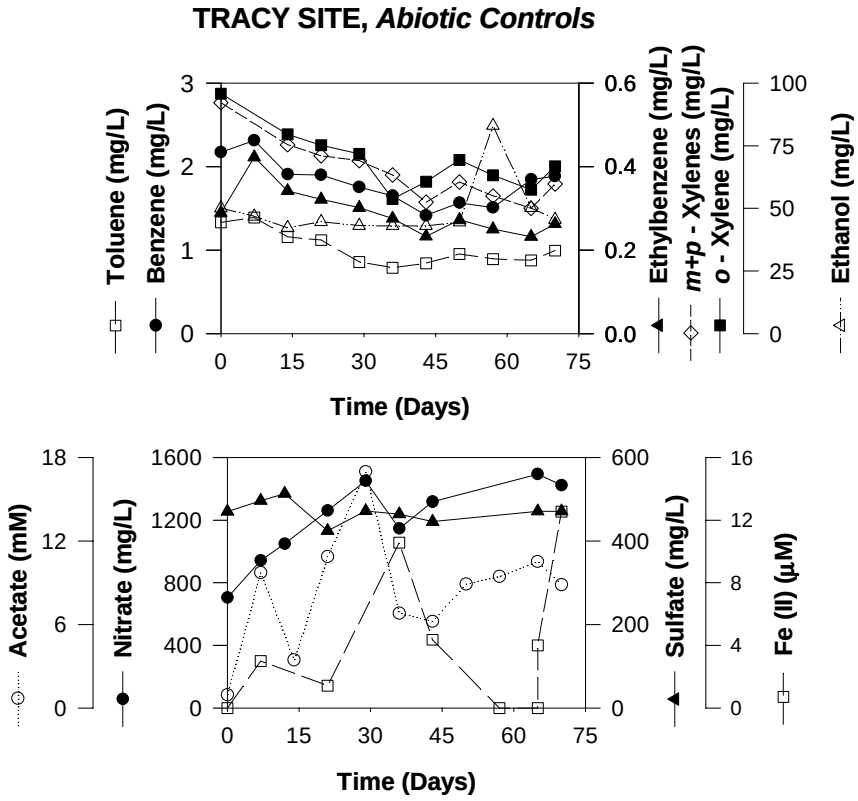


TRACY SITE, *Sulfate-reducing Conditions*



TRACY SITE, *"Methanogenic" Conditions*

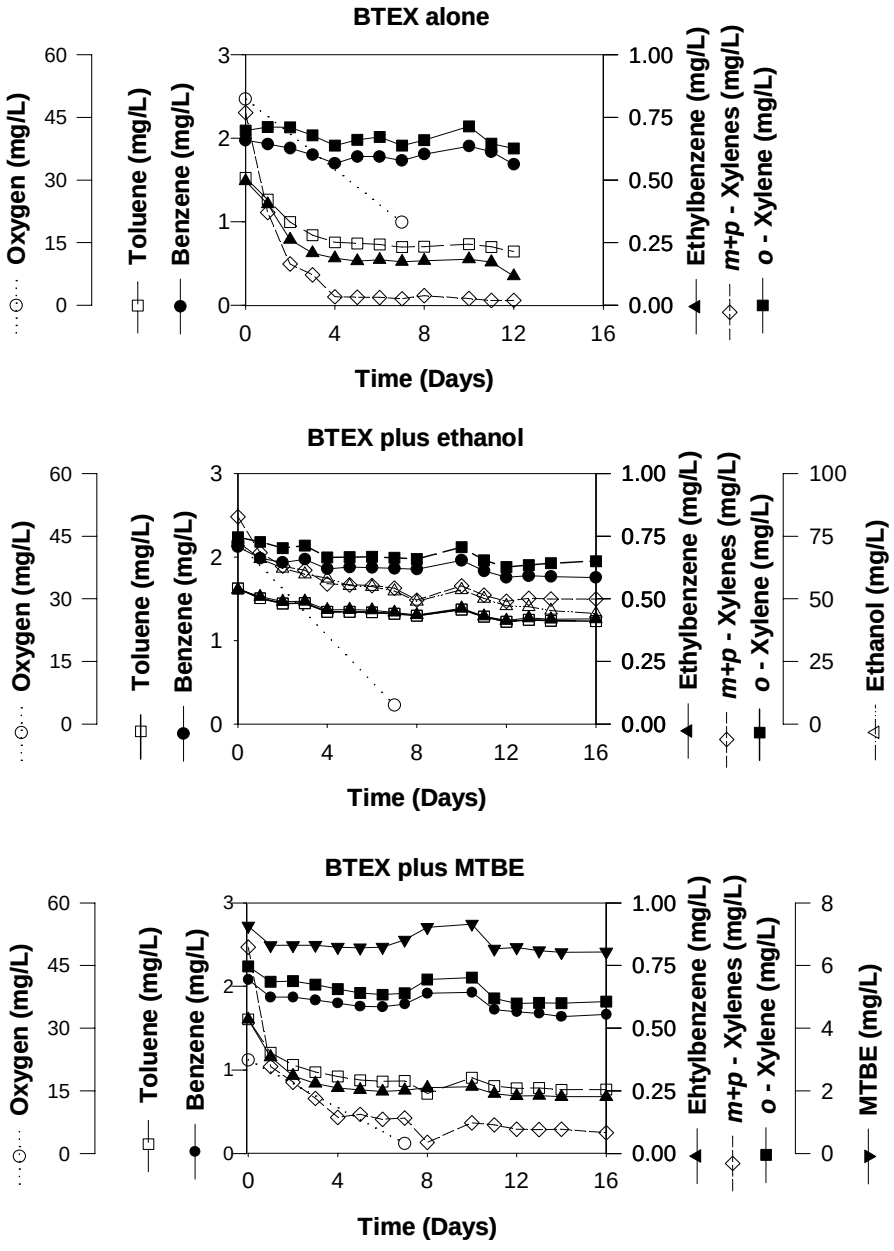




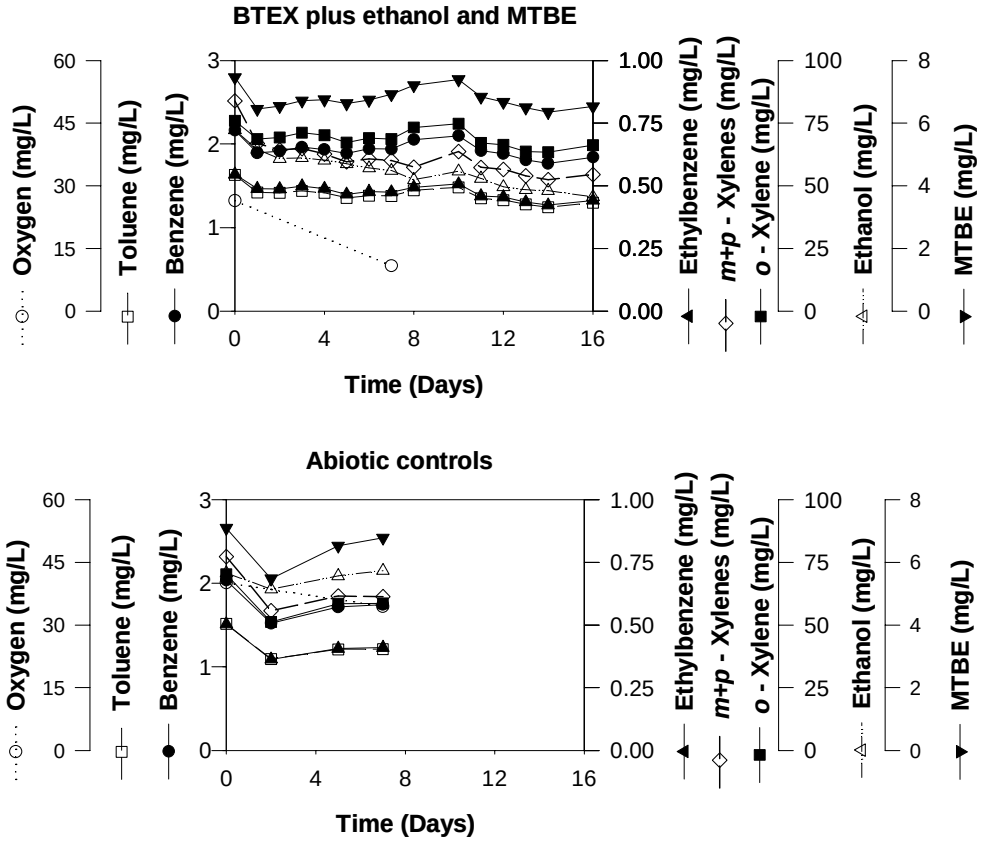
Note that it was difficult to maintain the controls sterile, as suggested by decreasing BTEX concentrations. Difficulties to keep soil sterile are commonly reported in the literature (e.g., Fava et al., 1998), where heat, $HgCl_2$ and NaN_3 have tested without complete success. Repeated poisoning with a the Kathon biocide partially alleviated this problem. The nitrate concentration increased during incubation period since Kathon contains magnesium nitrate.

Appendix C
Sacramento Site Microcosm Data

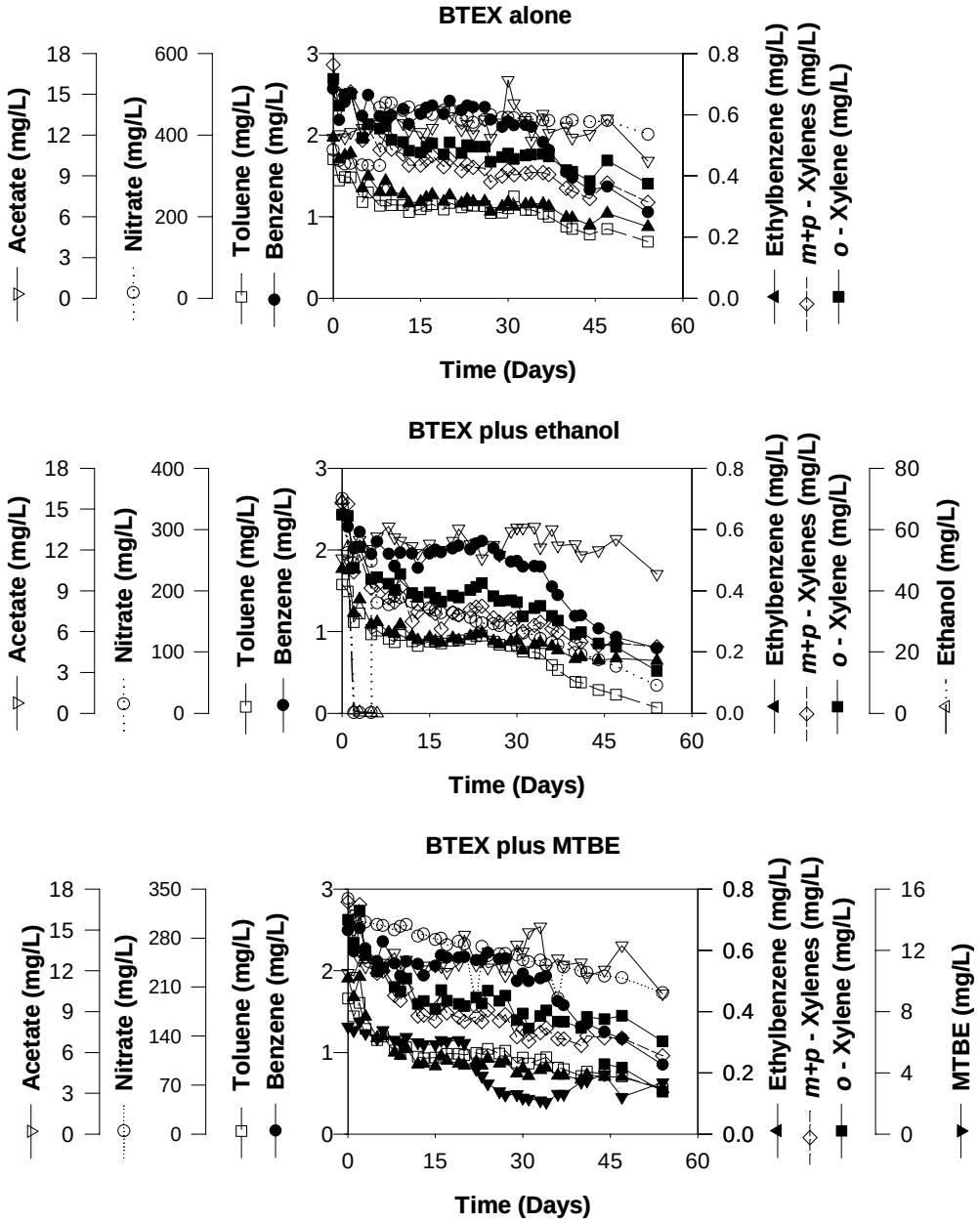
SACRAMENTO SITE, *Aerobic Conditions*



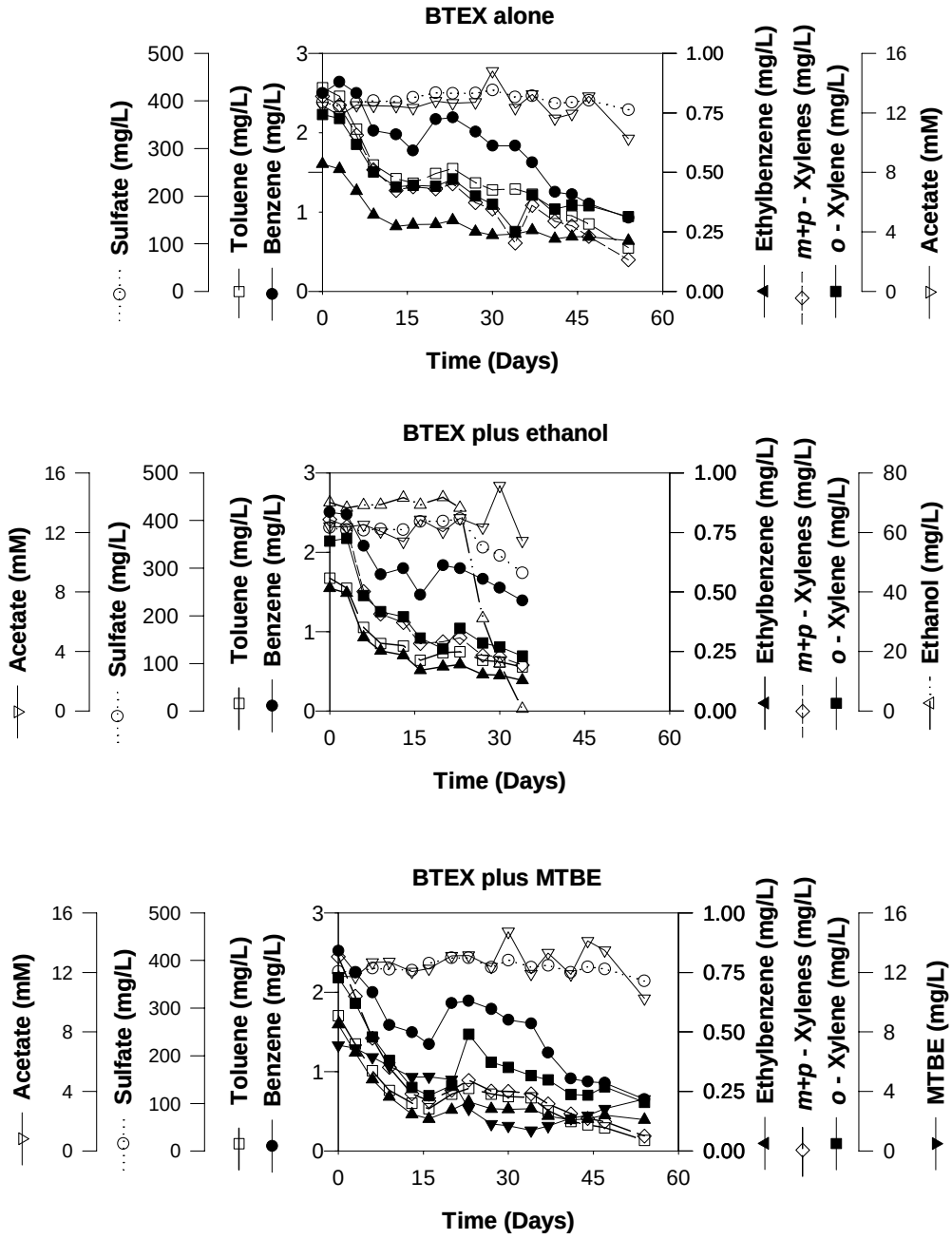
SACRAMENTO SITE, *Aerobic Conditions*



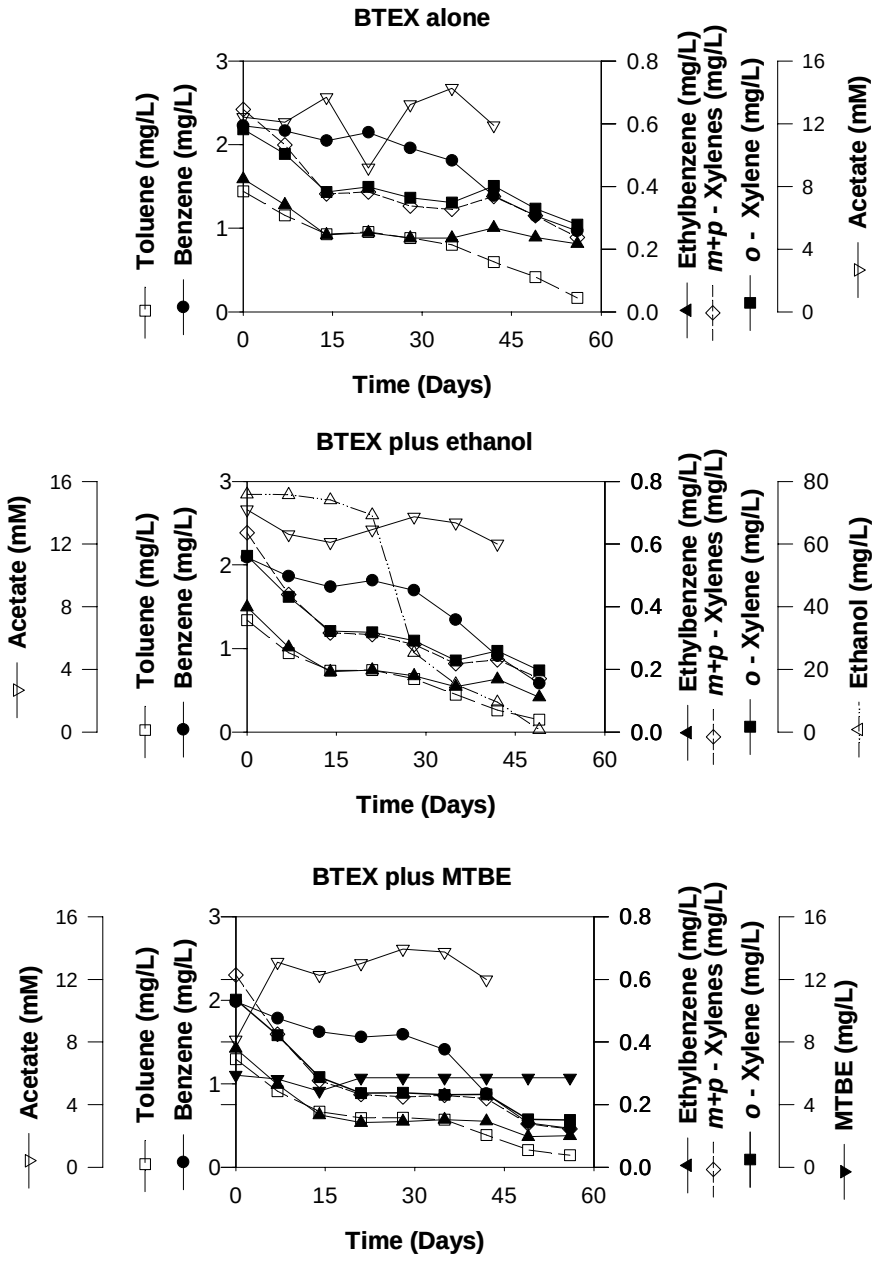
SACRAMENTO SITE, *Denitrifying Conditions*



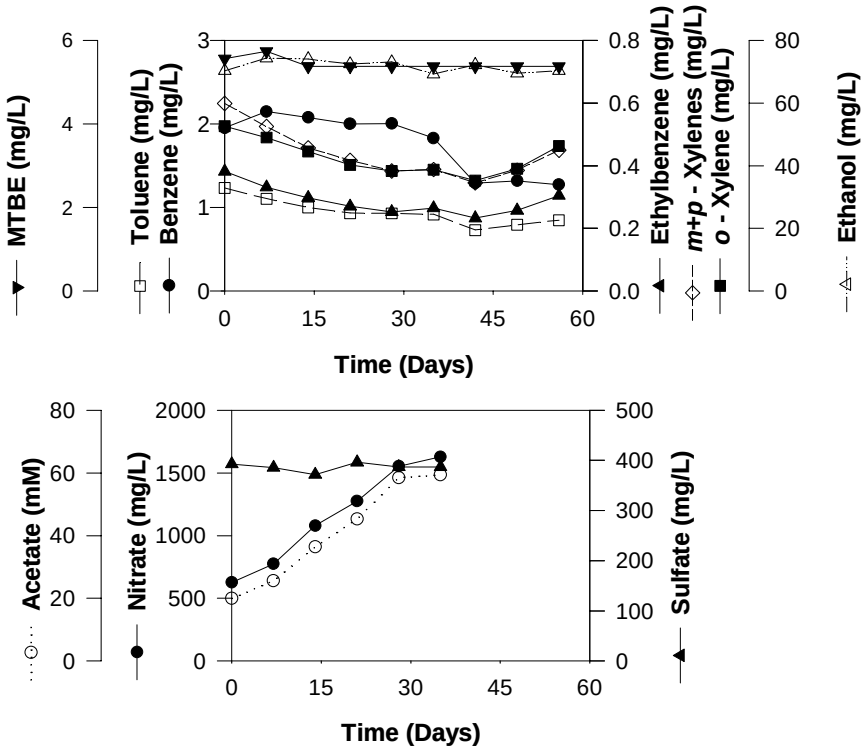
SACRAMENTO SITE, *Sulfate-reducing Conditions*



SACRAMENTO SITE, *"Methanogenic" Conditions*

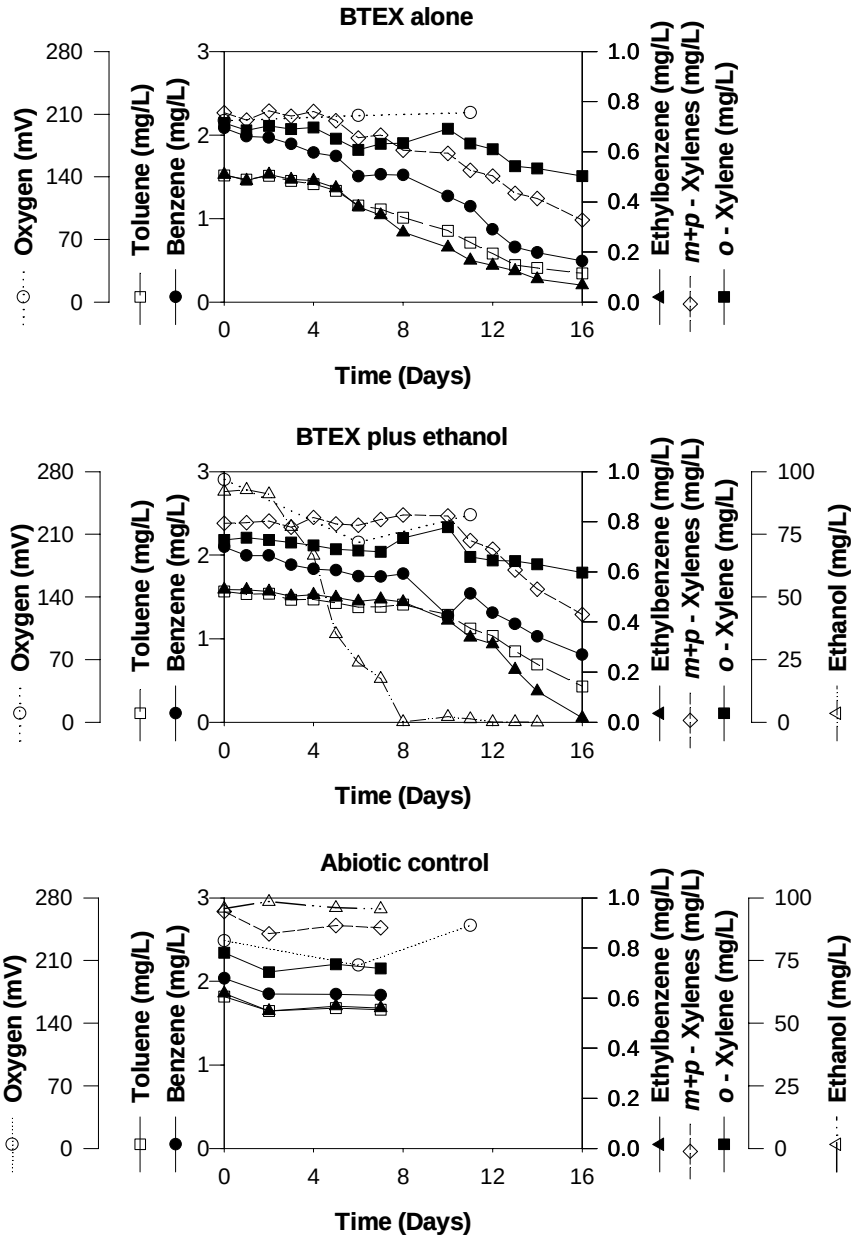


SACRAMENTO SITE, *Abiotic Controls*

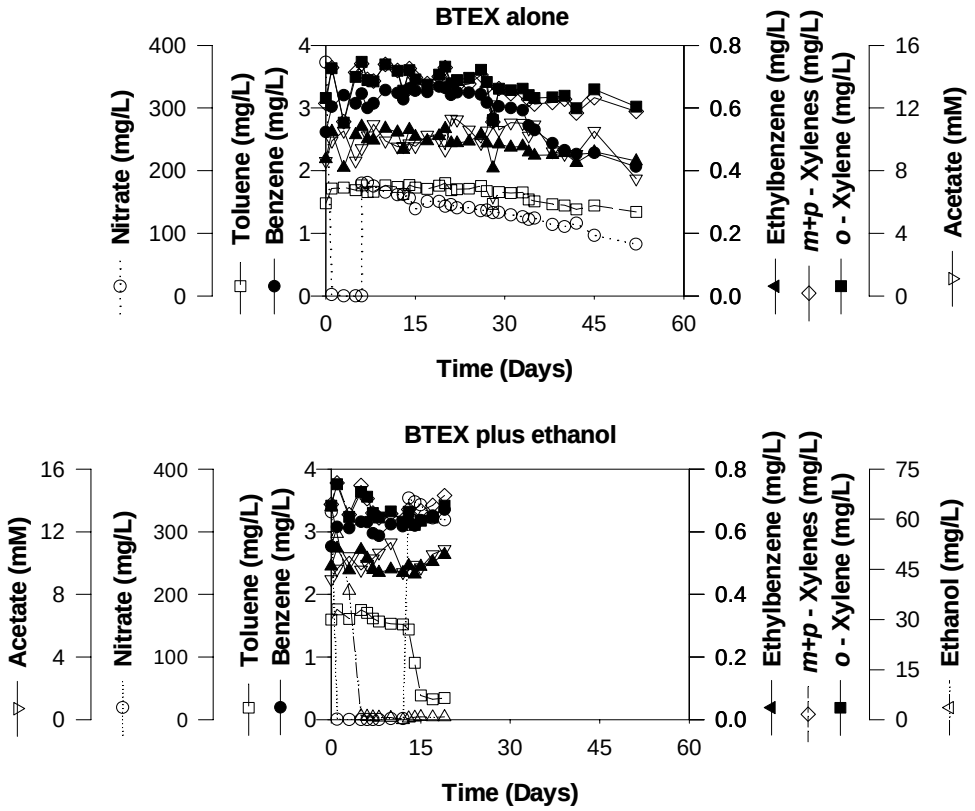


Appendix D
Northwest Terminal Site Microcosm Data

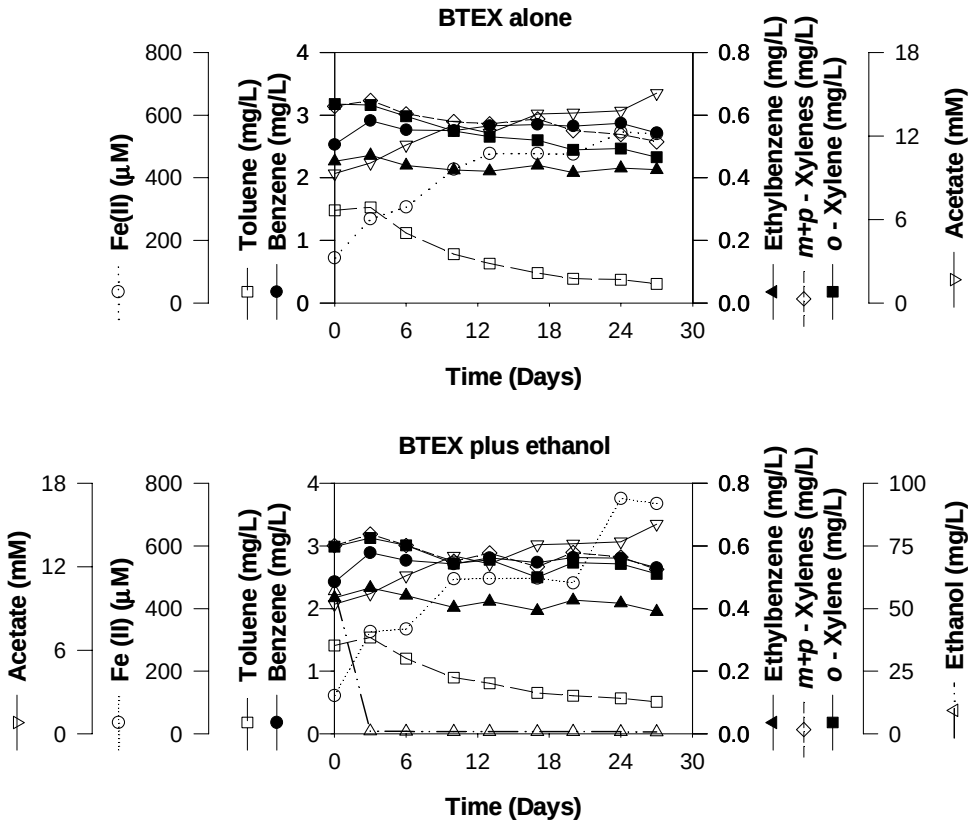
NORTHWEST TERMINAL SITE, *Aerobic conditions*



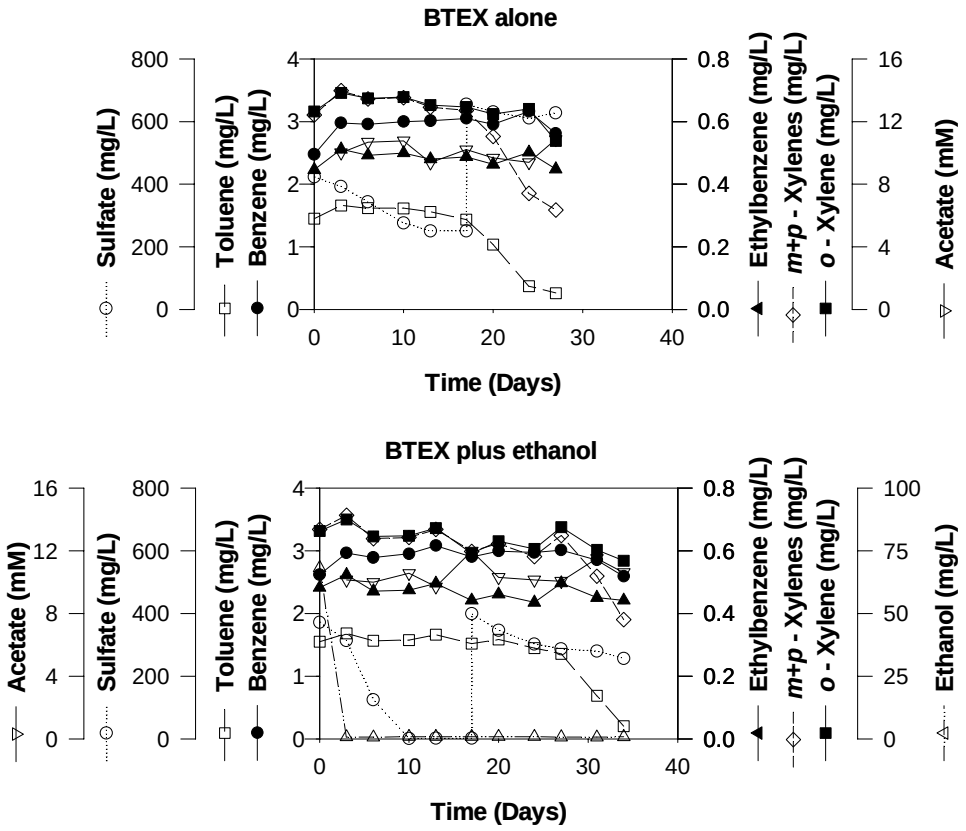
NORTHWEST TERMINAL SITE, *Dentrifying Conditions*



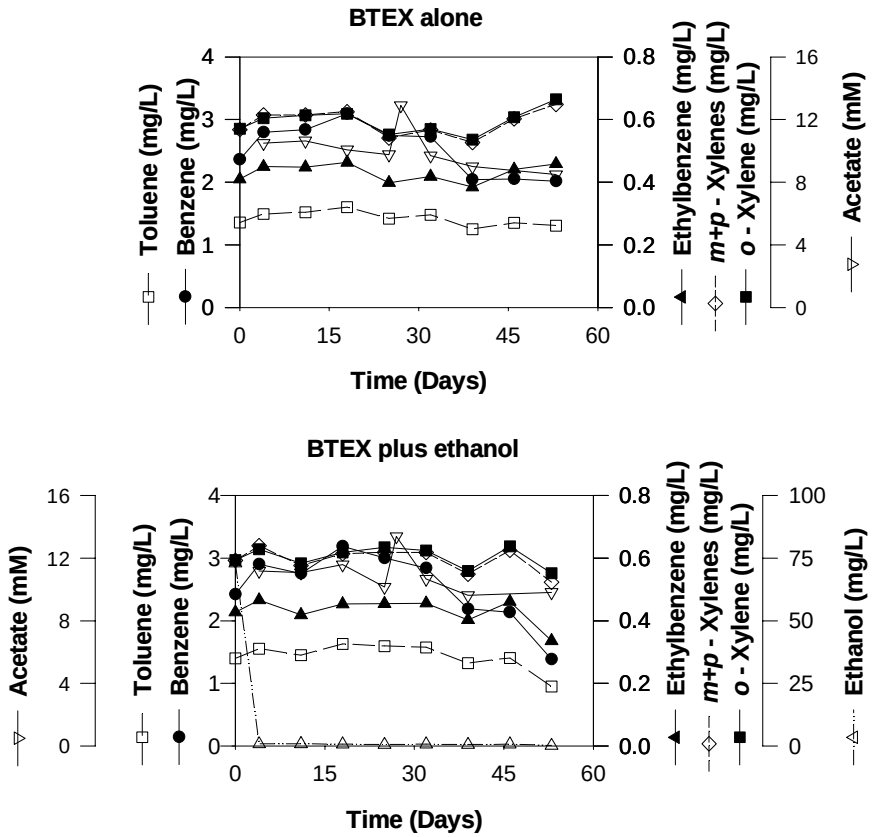
NORTHWEST TERMINAL SITE, *Iron (III)-amended Conditions*



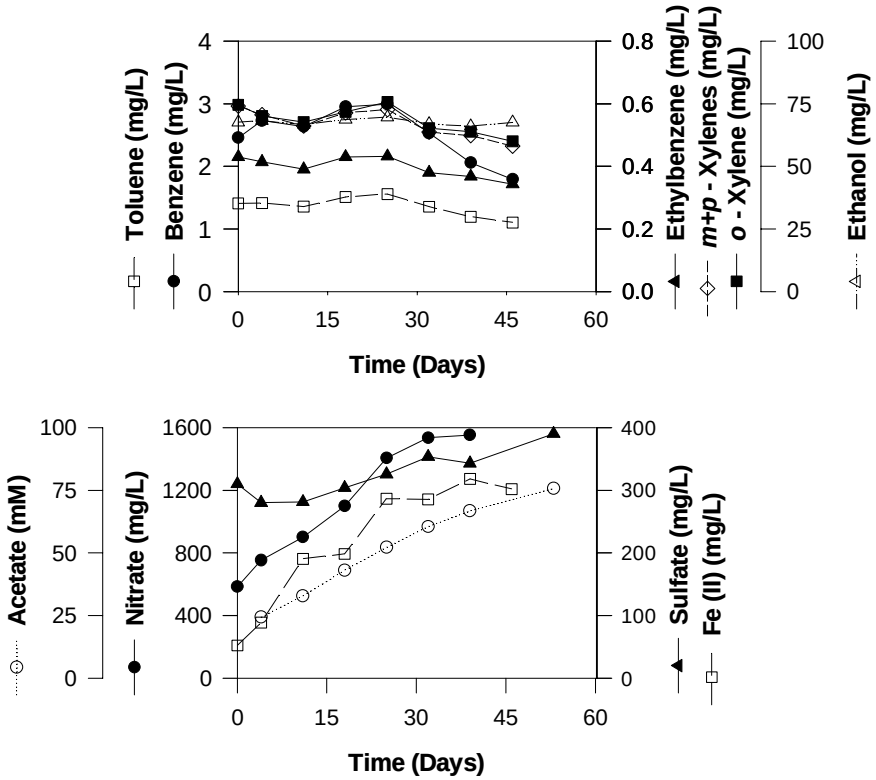
NORTHWEST TERMINAL SITE, *Sulfate-reducing Conditions*



NORTHWEST TERMINAL SITE, *"Methanogenic" Conditions*



NORTHWEST TERMINAL SITE, *Abiotic Controls*



Note that it was difficult to maintain the controls sterile, as suggested by decreasing BTEX concentrations. Difficulties to keep soil sterile are commonly reported in the literature (e.g., Fava et al., 1998), where heat, $HgCl_2$ and NaN_3 have tested without complete success. Repeated poisoning with a the Kathon biocide partially alleviated this problem. The nitrate concentration increased during incubation period since Kathon contains magnesium nitrate.

Appendix E
Incubation Times for Sacrificial Microcosms and Compounds Degraded at Time of Sampling

Travis AFB site

Conditions	Set	Replicate	Time (Days)	Compound degraded
Initial	**11**	**ABCD**	**0**	**NA**
Aerobic				
w/o ethanol	**1**	**AB**	**9**	**ND**
w/o ethanol	**1**	**CD**	**2**	**Ethylbenzene, Toluene**
w ethanol	**2**	**AB**	**9**	**Ethanol**
w ethanol	**2**	**CD**	**1**	**Ethylbenzene, Toluene**
Denitrifying				
w/o ethanol	**3**	**AB**	**12**	**Ethylbenzene**
w/o ethanol	**3**	**CD**	**5**	**Toluene, Ethylbenzene**
w ethanol	**4**	**AB**	**4**	**Ethanol**
w ethanol	**4**	**CD**	**12**	**Toluene**
Iron(III)-amended				
w/o ethanol	**5**	**AB**	**6**	**ND**
w/o ethanol	**5**	**CD**	**13**	**Toluene**
w ethanol	**6**	**AB**	**6**	**Ethanol**
w ethanol	**6**	**CD**	**37**	***m+p*** **- Xylenes**
Sulfate-reducing				
w/o ethanol	**7**	**AB**	**9**	**ND**
w/o ethanol	**7**	**CD**	**33**	***m+p*** **- Xylenes**
w ethanol	**8**	**AB**	**9**	**Ethanol**
w ethanol	**8**	**CD**	**12**	**Toluene**
"Methanogenic"				
w/o ethanol	**9**	**AB**	**7**	**ND**
w/o ethanol	**9**	**CD**	**21**	**Toluene**
w ethanol	**10**	**AB**	**7**	**Ethanol**
w ethanol	**10**	**CD**	**49**	**Toluene**

NA = Not added.

ND = No degradation of any BTEX compound.

Tracy site

Conditions	Set	Replicate	Time (Days)	Compound degraded
Initial	**11**	**ABCD**	**0**	**NA**
Aerobic				
w/o ethanol	**1**	**AB**	**7**	**ND**
w/o ethanol	**1**	**CD**	**9**	**Toluene, benzene**
w ethanol	**2**	**AB**	**7**	**Ethanol**
w ethanol	**2**	**CD**	**10**	**Benzene, toluene**
Denitrifying				
w/o ethanol	**3**	**AB**	**4**	**ND**
w/o ethanol	**3**	**CD**	**42**	**Toluene**
w ethanol	**4**	**AB**	**4**	**Ethanol**
w ethanol	**4**	**CD**	**51**	**Toluene**
Iron(III)-amended				
w/o ethanol	**5**	**AB**	**15**	**ND**
w/o ethanol	**5**	**CD**	**45**	**Toluene**
w ethanol	**6**	**AB**	**15**	**Ethanol**
w ethanol	**6**	**CD**	**70**	**ND**
Sulfate-reducing				
w/o ethanol	**7**	**AB**	**12**	**ND**
w/o ethanol	**7**	**CD**	**70**	**ND**
w ethanol	**8**	**AB**	**12**	**Ethanol**
w ethanol	**8**	**CD**	**70**	**ND**
"Methanogenic"				
w/o ethanol	**9**	**AB**	**14**	**ND**
w/o ethanol	**9**	**CD**	**70**	**ND**
w ethanol	**10**	**AB**	**14**	**Ethanol**
w ethanol	**10**	**CD**	**70**	**Ethylbenzene**

NA = Not added.

ND = No degradation of any BTEX compound.

Sacramento site

Conditions	Set	Replicate	Time (Days)	Compound degraded
Initial	**11**	**ABCD**	**0**	**NA**
Aerobic				
w/o ethanol	**1**	**AB**	**8**	**ND**
w/o ethanol	**1**	**CD**	**3**	**m+p -Xylenes**
w ethanol	**2**	**AB**	**8**	**Ethanol**
w ethanol	**2**	**CD**	**16**	**ND**
Denitrifying				
w/o ethanol	**3**	**AB**	**3**	**ND**
w/o ethanol	**3**	**CD**	**54**	**ND**
w ethanol	**4**	**AB**	**3**	**Ethanol**
w ethanol	**4**	**CD**	**47**	**Toluene**
Sulfate-reducing				
w/o ethanol	**5**	**AB**	**34**	**ND**
w/o ethanol	**5**	**CD**	**54**	**m+p – Xylenes**
w ethanol	**6**	**AB**	**34**	**Ethanol**
w ethanol	**6**	**CD**	**20**	**Ethylbenzene**
"Methanogenic"				
w/o ethanol	**7**	**AB**	**42**	**ND**
w/o ethanol	**7**	**CD**	**54**	**Toluene**
w ethanol	**8**	**AB**	**42**	**Ethanol**
w ethanol	**8**	**CD**	**42**	**Toluene**

NA = Not added.

ND = No degradation of any BTEX compound.

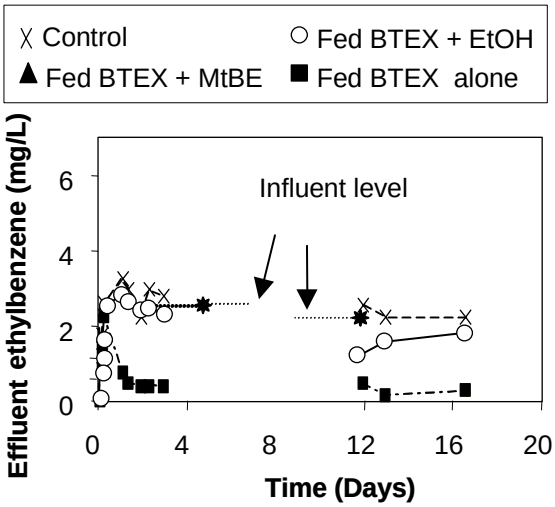
Northwest Terminal site

Conditions	Set	Replicate	Time (Days)	Compound degraded
Initial	**11**	**ABCD**	**0**	**NA**
Aerobic				
w/o ethanol	**1**	**AB**	**8**	**ND**
w/o ethanol	**1**	**CD**	**16**	**Ethylbenzene**
w ethanol	**2**	**AB**	**8**	**Ethanol**
w ethanol	**2**	**CD**	**16**	**Ethylbenzene**
Denitrifying				
w/o ethanol	**3**	**AB**	**5**	**ND**
w/o ethanol	**3**	**CD**	**50**	**ND**
w ethanol	**4**	**AB**	**5**	**Ethanol**
w ethanol	**4**	**CD**	**17**	**Toluene**
Iron (III)-amended				
w/o ethanol	**5**	**AB**	**3**	**ND**
w/o ethanol	**5**	**CD**	**24**	**Toluene**
w ethanol	**6**	**AB**	**3**	**Ethanol**
w ethanol	**6**	**CD**	**24**	**Toluene**
Sulfate-reducing				
w/o ethanol	**7**	**AB**	**3**	**ND**
w/o ethanol	**7**	**CD**	**24**	**Toluene**
w ethanol	**8**	**AB**	**3**	**Ethanol**
w ethanol	**8**	**CD**	**34**	**Toluene**
"Methanogenic"				
w/o ethanol	**9**	**AB**	**4**	**ND**
w/o ethanol	**9**	**CD**	**56**	**ND**
w ethanol	**10**	**AB**	**4**	**Ethanol**
w ethanol	**10**	**CD**	**56**	**ND**

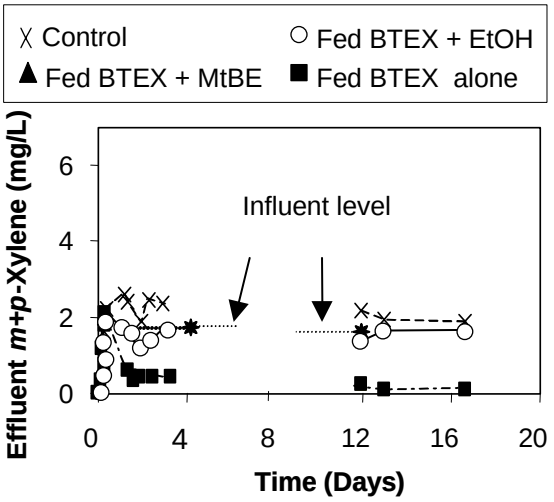
NA = Not added.

ND = No degradation of any BTEX compound.

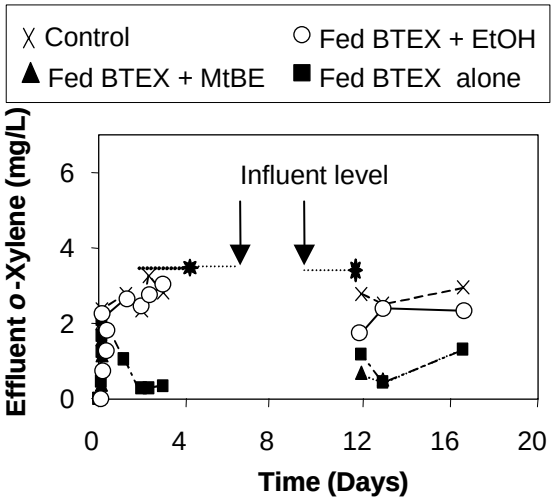
Appendix F
Breakthrough Curves from Non-sterile Columns



Ethylbenzene breakthrough from aquifer columns fed BTEX alone, with ethanol (150 mg/L) or with MTBE (12 mg/L). The columns were operated with a hydraulic retention time of 2.66 h.



m+*p*-Xylenes breakthrough from aquifer columns fed BTEX alone, with ethanol (150 mg/L) or with MTBE (12 mg/L). The columns were operated with a hydraulic retention time of 2.66 h.



o-Xylene breakthrough from aquifer columns fed BTEX alone, with ethanol (150 mg/L) or with MTBE (12 mg/L). The columns were operated with a hydraulic retention time of 2.66 h.